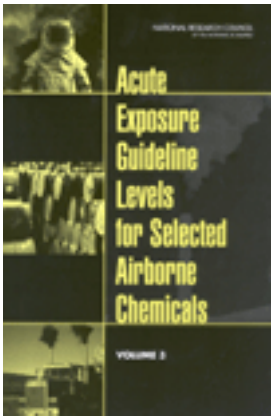

Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 3

Subcommittee on Acute Exposure Guideline Levels, Committee on Toxicology, National Research Council

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Acute Exposure Guideline Levels for Selected Airborne Chemicals

VOLUME 3

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Preface

Extremely hazardous substances (EHSs)[1] can be released accidentally as a result of chemical spills, industrial explosions, fires, or accidents involving railroad cars and trucks transporting EHSs. The people in communities surrounding industrial facilities where EHSs are manufactured, used, or stored and in communities along the nation's railways and highways are potentially at risk of being exposed to airborne EHSs during accidental releases. Pursuant to the Superfund Amendments and Reauthorization Act of 1986, the U.S. Environmental Protection Agency (EPA) has identified approximately 400 EHSs on the basis of acute lethality data in rodents.

As part of its efforts to develop acute exposure guideline levels for EHSs, EPA and the Agency for Toxic Substances and Disease Registry (ATSDR) in 1991 requested that the National Research Council (NRC) develop guidelines for establishing such levels. In response to that request, the NRC published *Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances* in 1993.

Using the 1993 NRC guidelines report, the National Advisory Committee (NAC) on Acute Exposure Guideline Levels for Hazardous Substances—consisting of members from EPA, the Department of Defense (DOD), the Department of Energy (DOE), the Department of Transportation, other federal and state governments, the chemical industry,

[1]As defined pursuant to the Superfund Amendments and Reauthorization Act of 1986.

academia, and other organizations from the private sector—has developed acute exposure guideline levels (AEGLs) for approximately 80 EHSs.

In 1998, EPA and DOD requested that the NRC independently review the AEGLs developed by NAC. In response to that request, the NRC organized within its Committee on Toxicology the Subcommittee on Acute Exposure Guideline Levels, which prepared this report. This report is the third volume in the series *Acute Exposure Guideline Levels for Selected Airborne Chemicals*. It reviews the AEGLs for the nerve agents (GA [tabun], GB [sarin], GD [soman], GF, and VX), sulfur mustard, diborane, and methyl isocyanate for scientific accuracy, completeness, and consistency with the NRC guideline reports.

This report was reviewed in draft by individuals selected for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report: Mohamed Abou-Donia of Duke University; Janice Chambers of Mississippi State University; and Sidney Green of Howard University.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by David Moore of Battelle Memorial Institute, appointed by the Division on Earth and Life Studies, who was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

The subcommittee gratefully acknowledges the valuable assistance provided by the following persons: Roger Garrett (deceased, March 31, 2003), Paul Tobin, and Ernest Falke (all from EPA); George Rusch (Honeywell, Inc.); Po Yung Lu, Claudia Troxel, Robert Young, Carol Forsyth, Dennis Opresko, and Annetta Watson (all from Oak Ridge National Laboratory). Aida Neel was the project assistant. Kelly Clark

edited the report. We are grateful to James J.Reisa, director of the Board on Environmental Studies and Toxicology (BEST), for his helpful comments. The subcommittee particularly acknowledges Kulbir Bakshi, project director for the subcommittee, for bringing the report to completion. Finally, we would like to thank all members of the subcommittee for their expertise and dedicated effort throughout the development of this report.

Daniel Krewski, *Chair*
Subcommittee on Acute Exposure
Guideline Levels
Bailus Walker, *Chair*
Committee on Toxicology

Dedication

The subcommittee dedicates this series of reports to our late colleague and director of the Acute Exposure Guideline Levels program, Dr. Roger L.Garrett, whose 27 years of distinguished service with the U.S. Environmental Protection Agency in the fields of toxicology and health-risk assessment contributed significantly to scientific knowledge, to the development of the Acute Exposure Guideline Levels program, and to the protection of public health and safety.

Contents

Acute Exposure Guideline Levels for Selected Airborne Chemicals

Volume 3

Introduction

This report is the third volume in the series *Acute Exposure Guideline Levels for Selected Airborne Chemicals*.

In the Bhopal disaster of 1984, approximately 2,000 residents living near a chemical plant were killed and 20,000 more suffered irreversible damage to their eyes and lungs following accidental release of methyl isocyanate. The toll was particularly high because the community had little idea what chemicals were being used at the plant, how dangerous they might be, and what steps to take in case of emergency. This tragedy served to focus international attention on the need for governments to identify hazardous substances and to assist local communities in planning how to deal with emergency exposures.

In the United States, the Superfund Amendments and Reauthorization Act (SARA) of 1986 required that the U.S. Environmental Protection Agency (EPA) identify extremely hazardous substances (EHSs) and, in cooperation with the Federal Emergency Management Agency and the Department of Transportation, assist Local Emergency Planning Committees (LEPCs) by providing guidance for conducting health-hazard assessments for the development of emergency-response plans for sites where EHSs are produced, stored, transported, or used. SARA also required that the Agency for Toxic Substances and Disease Registry (ATSDR) determine whether chemical substances identified at hazardous waste sites or in the environment present a public-health concern.

As a first step in assisting the LEPCs, EPA identified approximately 400 EHSs largely on the basis of their "immediately dangerous to life and health" (IDLH) values developed by the National Institute for Occupational Safety and Health (NIOSH) in experimental animals. Although several public and private groups, such as the Occupational Safety and Health Administration (OSHA) and the American Conference of Governmental Industrial Hygienists (ACGIH), have established exposure limits for some substances and some exposures (e.g., workplace or ambient air quality), these limits are not easily or directly translated into emergency exposure limits for exposures at high levels but of short duration, usually less than 1 h, and only once in a lifetime for the general population, which includes infants, children, the elderly, and persons with diseases, such as asthma, heart disease, or lung disease.

The National Research Council (NRC) Committee on Toxicology (COT) has published many reports on emergency exposure guidance levels and spacecraft maximum allowable concentrations for chemicals used by the Department of Defense (DOD) and the National Aeronautics and Space Administration (NASA) (NRC 1968, 1972, 1984a,b,c,d, 1985a,b, 1986a,b, 1987, 1988, 1994, 1996a,b, 2000). COT has also published guidelines for developing emergency exposure guidance levels for military personnel and for astronauts (NRC 1986b, 1992). Because of COT's experience in recommending emergency exposure levels for short-term exposures, in 1991 EPA and ATSDR requested that COT develop criteria and methods for developing emergency exposure levels for EHSs for the general population. In response to that request, the NRC assigned this project to the COT Subcommittee on Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances. The report of that subcommittee, *Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances* (NRC 1993), provides step-by-step guidance for setting emergency exposure levels for EHSs. Guidance is given on what data are needed, what data are available, how to evaluate the data, and how to present the results.

In November 1995, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC)[1] was established to identify, review, and interpret relevant toxicologic and other scientific data and to develop acute exposure guideline levels (AEGLs) for high-

[1]NAC is composed of members from EPA, DOD, many other federal and state agencies, industry, academia, and other organizations. The roster of NAC is shown on page 8.

priority, acutely toxic chemicals. The NRC's previous name for acute exposure levelsCcommunity emergency exposure levels (CEELs)Cwas replaced by the term AEGLs to reflect the broad application of these values to planning, response, and prevention in the community, the workplace, transportation, the military, and the remediation of Superfund sites.

Three levels—AEGL-1, AEGL-2, and AEGL-3—are developed for each of five exposure periods (10 min, 30 min, 1 h, 4 h, and 8 h) and are distinguished by varying degrees of severity of toxic effects.

The three AEGLs are defined as follows:

> AEGL-1 is the airborne concentration (expressed as ppm [parts per million] or mg/m^3 [milligrams per cubic meter]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic nonsensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.
>
> AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.
>
> AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below AEGL-1 represent exposure levels that can produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic, nonsensory adverse effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGL values represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to unique or idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

SUMMARY OF REPORT ON GUIDELINES FOR DEVELOPING AEGLS

As described in the *Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances* (NRC 1993) and the NAC guidelines report *Standing Operating Procedures on Acute Exposure Guideline Levels for Hazardous Substances* (NRC 2001), the first step in establishing AEGLs for a chemical is to collect and review all relevant published and unpublished information available on a chemical. Various types of evidence are assessed in establishing AEGL values for a chemical. These include information from (1) chemical-physical characterizations, (2) structure-activity relationships, (3) in vitro toxicity studies, (4) animal toxicity studies, (5) controlled human studies, (6) observations of humans involved in chemical accidents, and (7) epidemiologic studies. Toxicity data from human studies are most applicable and are used when available in preference to data from animal studies and in vitro studies. Toxicity data from inhalation exposures are most useful for setting AEGLs for airborne chemicals because inhalation is the most likely route of exposure and because extrapolation of data from other routes would lead to additional uncertainty in the AEGL estimate.

For most chemicals, actual human toxicity data are not available or critical information on exposure is lacking, so toxicity data from studies conducted in laboratory animals are extrapolated to estimate the potential toxicity in humans. Such extrapolation requires experienced scientific judgment. The toxicity data from animal species most representative of humans in terms of pharmacodynamic and pharmacokinetic properties are used for determining AEGLs. If data are not available on the species that best represents humans, the data from the most sensitive animal species are used to set AEGLs. Uncertainty factors are commonly used when animal data are used to estimate risk levels for humans. The magnitude of uncertainty factors depends on the quality of the animal data used to determine the no-observed-adverse-effect level (NOAEL) and the mode of action of the substance in question. When available, pharmacokinetic data on tissue doses are considered for interspecies extrapolation.

For substances that affect several organ systems or have multiple effects, all end points—including reproductive (in both sexes), develop-mental, neurotoxic, respiratory, and other organ-related effects—are evaluated, the most important or most sensitive effect receiving the greatest attention. For carcinogenic chemicals, excess carcinogenic risk is estimated, and the AEGLs corresponding to carcinogenic risks of 1 in 10,000 (1×10^{-4}), 1 in

100,000 (1×10^{-5}), and 1 in 1,000,000 (1×10^{-6}) exposed persons are estimated.

REVIEW OF AEGL REPORTS

As NAC began developing chemical-specific AEGL reports, EPA and DOD asked the NRC to review independently the NAC reports for their scientific validity, completeness, and consistency with the NRC guideline reports (NRC 1993; NRC in press). The NRC assigned this project to the COT Subcommittee on Acute Exposure Guideline Levels. The subcommittee has expertise in toxicology, epidemiology, pharmacology, medicine, industrial hygiene, biostatistics, risk assessment, and risk communication.

The AEGL draft reports are initially prepared by ad hoc AEGL Development Teams consisting of a chemical manager, two chemical reviewers, and a staff scientist of the NAC contractor—Oak Ridge National Laboratory. The draft documents are then reviewed by NAC and elevated from "draft" to "proposed" status. After the AEGL documents are approved by NAC, they are published in the *Federal Register* for public comment. The reports are then revised by NAC in response to the public comments, elevated from "proposed" to "interim" status, and sent to the NRC Subcommittee on Acute Exposure Guideline Levels for final evaluation.

The NRC subcommittee's review of the AEGL reports prepared by NAC and its contractors involves oral and written presentations to the subcommittee by the authors of the reports. The NRC subcommittee provides advice and recommendations for revisions to ensure scientific validity and consistency with the NRC guideline reports (NRC 1993, 2001). The revised reports are presented at subsequent meetings until the subcommittee is satisfied with the reviews.

Because of the enormous amount of data presented in the AEGL reports, the NRC subcommittee cannot verify all the data used by NAC. The NRC subcommittee relies on NAC for the accuracy and completeness of the toxicity data cited in the AEGLs reports.

This report is the third volume in the series *Acute Exposure Guideline Levels for Selected Airborne Chemicals.* AEGL documents for nerve agents (GA, GB, GD, GF, and VX), sulfur mustard, diborane, and methyl isocyanate are published as an appendix to this report. The subcommittee concludes that the AEGLs developed in those documents are scientifically valid conclusions based on the data reviewed by NAC and are consistent

with the NRC guideline reports. AEGL reports for additional chemicals will be presented in subsequent volumes.

REFERENCES

NRC (National Research Council). 1968. Atmospheric Contaminants in Spacecraft. Washington, DC: National Academy of Sciences.

NRC (National Research Council). 1972. Atmospheric Contaminants in Manned Spacecraft. Washington, DC: National Academy of Sciences.

NRC (National Research Council). 1984a. Emergency and Continuous Exposure Limits for Selected Airborne Contaminants, Vol. 1. Washington, DC: National Academy Press.

NRC (National Research Council). 1984b. Emergency and Continuous Exposure Limits for Selected Airborne Contaminants, Vol. 2. Washington, DC: National Academy Press.

NRC (National Research Council). 1984c. Emergency and Continuous Exposure Limits for Selected Airborne Contaminants, Vol. 3. Washington, DC: National Academy Press.

NRC (National Research Council). 1984d. Toxicity Testing: Strategies to Determine Needs and Priorities. Washington, DC: National Academy Press.

NRC (National Research Council). 1985a. Emergency and Continuous Exposure Guidance Levels for Selected Airborne Contaminants, Vol. 4. Washington, DC: National Academy Press.

NRC (National Research Council). 1985b. Emergency and Continuous Exposure Guidance Levels for Selected Airborne Contaminants, Vol. 5. Washington, DC: National Academy Press.

NRC (National Research Council). 1986a. Emergency and Continuous Exposure Guidance Levels for Selected Airborne Contaminants, Vol. 6. Washington, DC: National Academy Press.

NRC (National Research Council). 1986b. Criteria and Methods for Preparing Emergency Exposure Guidance Level (EEGL), Short-Term Public Emergency Guidance Level (SPEGL), and Continuous Exposure Guidance level (CEGL) Documents. Washington, DC: National Academy Press.

NRC (National Research Council). 1987. Emergency and Continuous Exposure Guidance Levels for Selected Airborne Contaminants, Vol. 7. Washington, DC: National Academy Press.

NRC (National Research Council). 1988. Emergency and Continuous Exposure Guidance Levels for Selected Airborne Contaminants, Vol. 8. Washington, DC: National Academy Press.

NRC (National Research Council). 1992. Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants . Washington, DC: National Academy Press.

NRC (National Research Council). 1993. Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances. Washington, DC: National Academy Press.

NRC (National Research Council). 1994. Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 1. Washington, DC: National Academy Press.

NRC (National Research Council). 1996a. Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 2. Washington, DC: National Academy Press.

NRC (National Research Council). 1996b. Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 3. Washington, DC: National Academy Press.

NRC (National Research Council). 2000. Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 4. Washington, DC: National Academy Press.

NRC (National Research Coiuncil) 2001. Acute Exposure Guideline Levels for Selected Airborne Chemicals. Washington, DC: National Academy Press.

NRC (National Research Council). 2001. Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Airborne Chemicals. Washington, DC: National Academy Press.

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Appendixes

1

Nerve Agents GA, GB, GD, GF, and VX[1]

SUMMARY

The nerve agents for which AEGL analyses have been performed include the G-series agents (GA [tabun], GB [sarin], GD [soman], and GF) and nerve agent VX. These agents are all toxic ester derivatives of phosphonic acid containing either a cyanide, fluoride, or sulfur substituent group; they are commonly termed "nerve" agents as a consequence of their anticholin

[1]This Document was prepared by the AEGLs Development Team comprising Annetta Watson, Dennis Opresko, and Robert Young (Oak Ridge National Laboratory) and John Hinz and Glenn Leach (Chemical Managers) of the National Advisory Committee (NAC) on Acute Exposure Guideline Levels for Hazardous Substances. The NAC reviewed and revised the document and the AEGL values as deemed necessary. Both the document and the AEGL values were then reviewed by the National Research Council (NRC) Subcommittee on Acute Exposure Guideline Levels. The NRC subcommittee concludes that the AEGLs developed in this document are scientifically valid conclusions based on the data reviewed by the NRC and are consistent with the NRC guidelines reports (NRC 1993, 2001).

esterase properties. These compounds were developed as chemical warfare agents, and one (agent GB, or sarin) was used by terrorists in the 1995 exposure incident that took place in the Tokyo subway system. The chemical names of these five agents are as follow: agent GA, dimethylamidocyanoethylphosphate (CAS Registry No. 77–81–6); agent GB, isopropyl methylphosphonofluoridate (CAS Registry No. 107–44–8); agent GD, pinacolyl methylphosphonofluoridate (CAS Registry No. 96–64–0); agent GF, O-cyclohexylmethyl-fluorophosphonate (CAS Registry No. 329–99–7); and agent VX, O-ethyl-S-(diisopropylaminoethyl) methyl phosphonothiolate (CAS Registry No. 50782–69–9).

The G agents are all viscous liquids of varying volatility (vapor density relative to air between 4.86 and 6.33) with faint odors ("faintly fruit," or "spicy," odor of camphor). Toxic effects may occur at vapor concentrations below those of odor detection. Agent VX is a amber-colored liquid with a vapor density of 9.2 (air=1) and is considered odorless. As a consequence, agent VX vapor possesses no olfactory warning properties.

The vapor pressures and acute toxicity of these agents are sufficiently high for the vapors to be rapidly lethal. Within the G-series, GB is considered a greater vapor hazard than agent GD. Agent GA represents a smaller vapor hazard and is expected to present a relevant contact hazard. The vapor density of agent GF is intermediate between that of agents GA and GD. Agent VX, which has a vapor density (9.2) greater that of any G agent under consideration, was deliberately formulated to possess a low volatility; VX is approximately 2,000 times less volatile than nerve agent GB (DA 1990). As a consequence, agent VX is a persistent, "terrain denial" military compound with the potential to off-gas toxic vapor for days following surface application.

Exposure to acutely toxic concentrations of nerve agents can result in excessive bronchial, salivary, ocular, and intestinal secretions and sweating, miosis, bronchospasm, intestinal hypermotility, bradycardia, muscle fasciculations, twitching, weakness, paralysis, loss of consciousness, convulsions, depression of the central respiratory drive, and death. Minimal effects observed at low vapor concentrations include miosis (contraction of the pupils of the eye, with subsequent decrease in pupil area), tightness of the chest, rhinorrhea, and dyspnea (Dunn and Sidell 1989).

The results of agent GB vapor exposure studies conducted with human volunteers indicate that the threshold for miosis and other minimal toxic effects falls in the range of 0.05–0.5 mg/m^3 for 10–30 minute (min) exposures. The findings are based on the results of low-concentration nerve agent exposures of informed volunteers who were under clinical supervi

sion during the periods of exposure as well as for postexposure periods of several months.

A concern associated with symptomatic exposures to anticholinesterase compounds such as the nerve agents is the possibility of chronic neurological effects. There is, at present, no evidence indicating that asymptomatic exposures to any of the nerve agents result in chronic neurological disorders. In general, the available epidemiological data indicate that most clinical signs of toxicity resolve within hours to days; severe miosis can require several months after exposure for resolution. However, several studies have shown that subclinical signs may persist for longer periods. Following the chemical terrorist attacks with nerve agent GB (sarin) that occurred in Japan in 1994 and 1995, clinical signs of agent toxicity were no longer apparent in the surviving victims 3 months (mo) after the exposures had occurred; however, several studies conducted on a small number of asymptomatic individuals 6–8 mo after the attack revealed subclinical signs of neurophysiological deficits as measured by event-related and visual evoked potentials, psychomotor performance, and increases in postural sway.

Small but measurable changes in single fibre electromyography (SFEMG) of the forearm were detectable between 4 and 15 mo following exposure to a concentration of agent GB that produced minimal clinical signs and symptoms in fully informed human subjects who were under clinical supervision in compliance with Helsinki accords (Baker and Sedgwick 1996). The SFEMG effects were not clinically significant and were not detectable after 15–30 mo. In a separate study of workers who had been occupationally exposed to agent GB (sarin), altered electroencephalograms (EEGs) were recorded 1 year (y) or more after the last exposure had occurred. Spectral analysis of the EEGs indicated significant increases in brain beta activity (12–30 Hz) in the exposed group when compared with nonexposed controls, and sleep EEGs revealed significantly increased rapid eye movement in the exposed workers; however, those observations were not clinically significant. Increases in beta activity were also observed in rhesus monkeys 1 y after being dosed with GB at 5 mg/kg. Slight, but nonsignificant, increases in beta activity, without deleterious effects on cognitive performance, were reported for marmosets injected with GB at 3.0 mg/kg and tested 15 mo later. The significance of subclinical neurological effects for the long-term health of exposed individuals has not been determined.

Animal data from vapor and oral exposure studies for the G-series nerve agents and agent VX suggest that agents GB and VX do not induce

reproductive or developmental effects in mammals. Oral exposure studies of agent GD in lab animals as well as injection exposure studies of agent GA likewise suggest a lack of reproductive or development effects for these agents. Neither agent GB nor agent VX were found to be genotoxic in a series of microbial and mammalian assays, but agent GA was reported to be weakly mutagenic. There is no evidence indicating that agents GB, GA, or VX are carcinogenic.

Derivation of G-Agent AEGL Estimates

The base of data for toxicological effects in humans is more complete for agent GB than for any of the other nerve agents under consideration in this analysis. Furthermore, agent GB is the only G agent for which sufficient human data are available to directly derive AEGL-1 and AEGL-2 estimates, and the only G agent for which sufficient laboratory animal data are available for deriving an AEGL-3 value for all five AEGL time periods.

AEGL-1 and AEGL-2 Values for G-series Agents

The AEGL-1 values for agent GB were derived from a well-conducted study on adult female Sprague-Dawley rats exposed whole-body in a dynamic airflow chamber to a range of GB vapor concentrations (0.01 to 0.48 mg/m^3) over three time durations (10 min, 60 min, or 240 min) (total of 283 agent-exposed rats of which 142 were female and 141 were male) (Mioduszewski et al. 2002b). With the inclusion of range-finding experiments and controls (N=130), a total of 423 rats were used in this well-conducted study, which involved highly credible protocols for GB vapor generation and measurement. Analysis of rat pupil diameters assessed pre-and postexposure allowed determination of EC_{50} values for miosis (defined as a postexposure pupil diameter of 50% or less of the preexposure diameter in 50% of the exposed population). Blood samples collected from tail vein and heart at 60 min and 7 d postexposure indicated no significant change from preexposure baseline in monitored blood RBC-ChE, butyrylcholinesterase (BuChE) or carboxylesterase. No other clinical signs were evident throughout the duration of the study. Gender differences (females more susceptible) were statistically significant at 10 min (p= 0.014) and 240 min (p=0.023), but not at 60 min (p=0.054). This is a

well-defined animal end point in a susceptible gender, and it is transient, reversible, and nondisabling.

In terms of potential effects on humans, an EC_{50} for miosis is not considered an adverse effect. This degree of miosis is the first measurable change, by modern and reproducible techniques, in the continuum of response to anticholinesterase compounds. In bright daylight or under bright lighting, a 50% reduction in pupil diameter would result in greater visual acuity among some members of the affected exposed population and no marked reduction in visual acuity for the majority of the affected population. In twilight or dim light conditions, 50% reduction in pupil diameter in some persons would result in reduced visual acuity and less-than-optimal performance of tasks requiring operation of vehicular controls, monitoring or tracking on computer screens, reading of fine text, or shifts in focus between near and far fields. For individuals with central cataracts, the effects would be more pronounced at all illumination levels. During the Tokyo Subway Incident (terrorist release of GB), persons experiencing ≥50% reduction in pupil diameter were able to self-rescue and to render aid to others.

Data from GB vapor studies of nonhuman primates (marmosets, 5 h exposures to GB vapor concentrations at 0.05 to 150 $\mu g/m^3$) (van Helden et al. 2001, 2002) and human volunteers (minimal and reversible effects of miosis, rhinorrhea, headache, etc., after a 20-min exposure to a GB vapor concentration at 0.05 mg/m^3) (Harvey 1952; Johns 1952) are considered secondary and supportive. The human data of Harvey (1952) and Johns (1952) indicate that some adult humans exposed to concentrations within the exposure range tested by Mioduszewski et al. (2002b) would experience some discomfort (headache, eye pain, nausea, etc.) in addition to miosis corresponding to ≤50% pupil area decrement but no disability (see definition of AEGL-1 provided in NRC [2001]). Compared to the available human data, the miosis data derived from the study on rats (Mioduszewski et al. 2002b) are considered a more reliable data set because they are based on current and multiple analytical techniques for quantifying exposures and measuring miosis and because they apply an experimental protocol incorporating sufficiently large test and control populations. With the additional knowledge that the EC_{50} exhibited by rats in the study of Mioduszewski et al. (2002b) is transient and reversible, the determination was made that EC_{50} for miosis in female (susceptible gender) SD rats is an appropriate end point for estimating AEGL-1 values. Mioduszewski et al. (2002b) is considered the critical study for derivation of AEGL-1 estimates for agent GB.

The weight-of-evidence analysis indicates reasonable concordance among AEGL-1 estimates derived from the female Sprague-Dawley rat, the marmoset, and the human data sets identified above. Application of the Mioduszewski et al. (2002b) rat miosis data did not significantly change the interim values for AEGL-1 (based on the human experimental data of Harvey [1952] and Johns [1952]) but confirmed that the interim values were representative, protective, and could be retained as final AEGL-1 values.

The AEGL-2 values for agent GB were derived from a study in which miosis, dyspnea, photophobia, inhibition of red blood cell cholinesterase (RBC-ChE), and changes in single fibre electromyography (SFEMG) were observed in human volunteers following a 30-min exposure at 0.5 mg/m^3 (Baker and Sedgwick 1996). The SFEMG changes noted in the study were not clinically significant and were not detectable after 15–30 mo. Baker and Sedgwick considered SFEMG changes a possible early indicator or precursor of the nondepolarising neuromuscular block associated with intermediate-syndrome paralysis in severe organophosphorous insecticide poisoning cases. They concluded that the electromyographic changes were persistent (>15 mo), but that they were reversible and subclinical.

Although not considered debilitating or permanent effects in themselves, SFEMG changes are considered an early indicator of exposures that potentially could result in more significant effects. Selection of this effect as a protective definition of an AEGL-2 level is considered appropriate given the steep dose-response toxicity curve of nerve agents (Aas et al. 1985; Mioduszewski et al. 2000, 2001, 2002a). The concept of added precaution for steep dose-response is consistent with the emergency planning guidance for nerve agents that was developed by the National Center for Environmental Health of the Centers for Disease Control and Prevention (Thacker 1994).

Animals exposed to low concentrations of the G agents exhibit the same signs of toxicity as humans, including miosis, salivation, rhinorrhea, dyspnea, and muscle fasciculations. Studies on dogs and rats indicate that exposures to GB at 0.001 mg/m^3 for up to 6 h/d are unlikely to produce any signs of toxicity.

Because exposure-response data were not available for all of the AEGL-specific exposure durations, temporal extrapolation was used in the development of AEGL values for some of the AEGL-specific time periods. The concentration-exposure time relationship for many systemically acting vapors and gases may be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5. The temporal extrapolation used here is based on

a log-log linear regression of the LC_{01} lethality of GB in female Sprague-Dawley rats (Mioduszewski et al. 2000, 2001, 2002a) and a log-log linear regression of female SD rat miosis data following GB vapor exposure for durations of 10–240 min (Mioduszewski et al. 2002b). Regression analysis of the LC_{01} values yields an n value of 1.93 with an r^2 of 0.9948, and regression analysis of the miosis data yields an n value of 2.00 with an r^2 of 0.4335 (24 data points; see Appendix B). Given that all mammalian toxicity end points observed in the data set for all nerve agents represent different points on the response continuum for anticholinesterase exposure, and that the mechanism of acute mammalian toxicity (cholinesterase inhibition) is the same for all nerve agents, the experimentally derived n=2 from the Mioduszewski et al. (2000, 2001, 2002a,b) rat lethality and miosis data sets is used as the scaling function for all the AEGL derivations rather than a default value. An n of 1.16 (r^2=0.6704) was calculated for comparison using other data (human volunteer) and other end points (e.g., GB-induced miosis in humans; see Appendix B). However, because of uncertainties associated with some of the exposure measurements in the earlier studies, the Mioduszewki et al. rat data were determined to be the best source of an estimate for n. The n value of 2 was used to extrapolate for exposure time periods for which there were no experimental data. Those included (1) the 8-h AEGL-3 value (extrapolated from experimental data for 6 h); (2) the 30-min and 8-h AEGL-1 values (extrapolated from 10-min and 4-h experimental data; and (3) all of the AEGL-2 values (extrapolated from experimental data for 30 min).

In consultation with experimental investigators at Porton Down (United Kingdom) and the TNO Prins Maurits Laboratory (Netherlands), the analysis has determined that the miotogenic response of mammalian eyes to agent GB vapor exposure is similar across species. The species evaluated include standard laboratory animals (rabbits, rats, guinea pigs), nonhuman primates (marmosets), and humans. As a consequence, the interspecies uncertainty factor (UF) for the critical AEGL-1 end point of miosis is considered equal to 1. To accommodate known variation in human cholinesterase and carboxylesterase activity that may make some individuals susceptible to the effects of cholinesterase inhibitors such as nerve agents, a factor of 10 was applied for intraspecies variability (protection of susceptible populations). A modifying factor is not applicable. Thus, the total UF for estimating AEGL-1 values for agent GB is 10.

The fact that AEGL-2 analyses for agent GB are based on data from human volunteers (Baker and Sedgwick 1996) precludes the use of an interspecies UF. As was the case in the AEGL-1 estimations, a factor of 10 was

applied for intraspecies variability (protection of susceptible populations). A modifying factor is not applicable. Thus, the total UF for estimating AEGL-2 values for agent GB is 10.

In comparison to the data set for agent GB, the data sets characterizing the toxicity of agents GA, GD, and GF are less complete. However, the database for the G agents as a group is considered reasonably complete in that there is/are (1) experimental data for multiple species, including humans; (2) documented nonlethal and lethal end points that follow an exposure-response curve; (3) a known mechanism of toxicity common to all the G agents with all end points representing a response continuum to inhibition of cholinesterase activity; and (4) no uncertainties regarding other toxic end points such as reproductive or developmental effects or carcinogenicity. Because the mechanism of action is the same for all the G agents, data uncertainty is reduced, and target organ effects are expected to be identical, but different in magnitude. Thus, it was possible to develop AEGL estimates for agents GA, GD, and GF by a comparative method of relative potency analysis from the more complete data set for agent GB. This concept has been applied before in the estimation of G-series nerve agent exposure limits, most recently by Mioduszewski et al. (1998).

The AEGL-1 and AEGL-2 values for agents GA, GD, and GF were derived from the AEGL-1 and AEGL-2 values for GB using a relative potency approach based on the potency of the agents needed to induce LOAEL effects of miosis, rhinorrhea, and SFEMG and agent concentration in milligrams per cubic meter. Agents GA and GB were considered to have an equivalent potency for causing miosis; thus, the AEGL-1 values for agents GA and GB are equal in milligrams per cubic meter. Agents GD and GF are considered approximately 2 times as potent as agents GB or GA for these end points, and equipotent to each other for AEGL-1 and AEGL-2 effects. Thus, the AEGL-1 and AEGL-2 concentration values for agents GD and GF are equal to 0.5 times those values derived for agents GA and GB, in milligrams per cubic meter.

AEGL-3 Values for G-Series Agents

AEGL-3 values for agent GB were derived from recent inhalation studies in which the lethality of GB vapor in female Sprague-Dawley rats was evaluated for 10-, 30-, 60-, 90-, 240-, and 360-min time periods (Mioduszewski et al. 2000, 2001, 2002a). Both experimental LC_{01} and LC_{50} values were evaluated. The use of a rat data set resulted in selection

of an interspecies UF of 3; the full default value of 10 was not considered appropriate because the mechanism of toxicity in rats and humans is the same, and lethality represents one point on the response continuum for these anticholinesterase compounds. The full default value of 10 for intraspecies uncertainty was considered necessary to protect susceptible populations. Because a modifying factor is not applicable, the composite UF for AEGL-3 determination for agent GB is equal to 30.

The AEGL-3 values for agent GA were derived from the AEGL-3 values for GB using a relative potency approach based on lethality of the agents; the potency of agent GA was considered to be only one-half that of agent GB for this end point. Thus, the AEGL-3 concentration values for agent GA are equal to 2.0 times the AEGL-3 values for agent GB, in milligrams per cubic meter.

The lethal potencies of agents GD and GF are considered equivalent and equipotent to that of agent GB; thus, the AEGL-3 concentration values for agent GB, GD, and GF are equal in milligrams per cubic meter, and the same composite UF (30) was applied in the derivation of the AEGL-3 values for agents GB, GD, and GF. For comparison, AEGL-3 values for GD were alternately derived from a secondary and short-term GD inhalation study of rat lethality for exposure times ≤30 min (Aas et al. 1985). As was the case in the derivation of the GB AEGLs, an *n* value of 2 was used for extrapolating to different time periods; however, because of the sparse data set for GD, the full default values for interspecies (10) and intraspecies (10) uncertainty were applied to the Aas et al. (1985) data. Because a modifying factor is not applicable, a composite UF of 100 was used for the Aas et al. (1985) data, whereas in the GB AEGL derivation from the Mioduszewski et al. (2000, 2001, 2002a) rat lethality data, a composite UF of 30 was used. The resulting 10-min AEGL-3 (0.27 mg/m^3) and 30-min AEGL-3 (0.15 mg/m^3) estimates for agent GD from Aas et al. (1985) are very similar to those for GB (0.38 mg/m^3 for 10 min and 0.19 mg/m^3 for 30 min) from Mioduszewski et al. (2000, 2001, 2002a) and support the assumption of lethal equipotency for agents GB and GD.

Derivation of Agent VX AEGL Estimates

Insufficient data are available from which to directly derive AEGL values for VX from human or animal inhalation toxicity studies. The few studies available are historical and are considered nonverifiable because of flawed study design, poor sampling techniques, or suspect contamination

of sampling and detection apparatus. Nevertheless, available literature clearly indicates that inhibition of cholinesterase activity is a common mechanism of toxicity shared by the G-series nerve agents and nerve agent VX. Thus, it was possible to develop AEGL estimates for agent VX by a comparative method of relative potency analysis from the more complete data set for nerve agent GB. The concept has been applied before in the estimation of agent VX exposure limits, most recently by Reutter et al. (2000). There are a number of estimates in the literature regarding the potency of VX relative to agent GB; all estimates indicate that vapor toxicity for agent VX is greater than that for agent GB. Comparable RBC-ChE_{50} data from clinically supervised human volunteers (Grob and Harvey 1958; Sidell and Groff 1974), who were exposed to agents GB and VX during well-conducted studies, are available for estimation of relative potency. The human data indicate that agent VX is approximately 4 times more potent than agent GB for inducing the RBC-ChE_{50} end point, which is considered an early and quantitative measure of the response continuum known for those compounds. Thus, the GB:VX relative potency ratio of 4 is considered an appropriate estimate of GB:VX relative potency for all VX AEGL determinations.

All mammalian toxicity end points observed in the data set for nerve agent VX as well as the G-series agents represent different points on the response continuum for anticholinesterase effects. Further, the mechanism of mammalian toxicity (cholinesterase inhibition) is the same for all nerve agents. In consequence, the experimentally derived n=2 from the Mioduszewski et al. (2000, 2001, 2002a,b) rat miosis and lethality data sets for agent GB are used as the scaling function for the agent-VX AEGL-1, AEGL-2, and AEGL-3 derivations rather than a default value.

By applying the GB:VX relative potency concept outlined above (the relative potency of GB:VX equal to 4), the AEGL-1 analyses for agent VX are derived from miosis data for adult female SD rats exposed to GB vapor for three time durations of significance for AEGLs (10, 60, and 240 min) (Mioduszewski et al. 2002b). Data from a GB vapor study of nonhuman primates (marmosets, 5 h exposures to GB vapor concentrations at 0.05–150 μg/m^3) (van Helden et al. 2001, 2002) and human volunteers (minimal and reversible effects of miosis, rhinorrhea, headache, etc., after a 20-min exposure to a GB vapor concentration at 0.05 mg/m^3) (Harvey 1952; Johns 1952) are considered secondary and supportive. The same UFs and logic applied in the derivation of AEGL-1 and AEGL-2 values for agent GB (e.g., interspecies UF of 1, intraspecies UF of 10) are used here for estimat

ing AEGL-1 and AEGL-2 values for agent VX. With application of a modifying factor of 3 for the sparse VX data set, the total UF for estimating AEGL-1 values for agent VX (from the GB data set of Mioduszewski et al. [2002b]) is 30.

By further application of the GB:VX relative potency concept outlined above, the AEGL-2 values for agent VX were derived from a GB vapor exposure study of human subjects in which miosis, dyspnea, photophobia, inhibition of red blood cell cholinesterase (RBC-ChE) to approximately 60% of individual baseline, and small but measurable changes in SFEMG of the forearm occurred following a 30-min exposure at 0.5 mg GB/m^3 (Baker and Sedgwick 1996).

The fact that AEGL-2 analyses for agent VX are based on data from clinically supervised human volunteers exposed to GB vapor (Baker and Sedgwick 1996) precludes the use of an interspecies UF. With application of a factor of 10 for intraspecies variability and a modifying factor of 3 for the sparse VX data set, the total UF for estimating AEGL-2 values for agent VX (from the GB data set of Baker and Sedgwick [1996]) is 30.

By further application of the GB:VX relative potency concept outlined above, the AEGL-3 values for agent VX were derived from recent inhalation studies in which the lethality of GB to female Sprague-Dawley rats was evaluated for the 10-, 30-, 60-, 90-, 240-, and 360-min time periods (Mioduszewski et al. 2000, 2001, 2002a). Both experimental LC_{01} and LC_{50} values were evaluated. The same UFs and logic applied in the derivation of AEGL-3 values for agent GB (interspecies UF of 3 and an intraspecies UF of 10) are used here for agent VX. With the additional application of a modifying factor of 3 for the sparse VX data set, the total UF for AEGL-3 determination for agent VX is equal to 100.

Research Needs

G-Series Agents

Further data analysis and experimentation is needed to more fully understand gender differences in susceptibility to nonlethal and lethal end points among the test population of SD rats. Interspecies susceptibility could be more fully characterized by determining if similar results can be obtained for the same protocol with different test species (particularly nonhuman primates).

The scarcity of dose-response data for agents GA, GD, and GF forces

the AEGL analysis to rely on assumptions of relative potency that need experimental confirmation.

Agent VX

It is noted that additional research to more fully characterize VX is needed in the following areas:

1. The toxicity of VX vapor in whole-animal systems. It is noted that specific experimental focus should be on obtaining data that would reduce uncertainties regarding the relative potency of agents GB and VX, or the potency of agent VX, for critical effects such as miosis, rhinorrhea, and lethality. Such studies could be adequately performed on a limited test population and scale.
2. The emissions profile expected during VX release, especially the generation and yield of VX vapors versus aerosol.
3. Comparative examination of agents GB and VX with regard to noncholinergic mechanisms in an effort to correlate whole-organism toxic responses with those reported for in vitro rat hippocampal cells in culture. The primary goal would be to generate a more refined determination of GB:VX relative potency.

Final AEGL estimates for the G-series nerve agents and VX are given in the summary table below.

1. INTRODUCTION

This evaluation of the AEGL values for the nerve agents GA, GB, GD, and GF is based on studies and data that are documented in the open literature as well as some unclassified documents with limited distribution requirements. Because of the military-specific nature of these compounds, some additional reports from the United States and elsewhere with classified or restricted distribution requirements exist. However, because of the open review process established by the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances, classified and other restricted-distribution reports are not cited in this evaluation, to the best of our knowledge.

TABLE 1-1 Summary of Final AEGL Values for Nerve Agents GA, GB, GD, GF, and VX[a]

Agent	Classification	10-min	30-min	1-h	4-h	8-h	End Point (Reference)
GA	AEGL-1 (Nondisabling)	0.0010 ppm (0.0069 mg/m^3)	0.00060 ppm (0.0040 mg/m^3)	0.00042 ppm (0.0028 mg/m^3)	0.00021 ppm (0.0014 mg/m^3)	0.00015 ppm (0.0010 mg/m^3)	Based on relative potency from GB[b]
	AEGL-2 (Disabling)	0.013 ppm (0.087 mg/m^3)	0.0075 ppm (0.050 mg/m^3)	0.0053 ppm (0.035 mg/m^3)	0.0026 ppm (0.017 mg/m^3)	0.0020 ppm (0.013 mg/m^3)	Based on relative potency from GB[b]
	AEGL-3 (Lethal)	0.11 ppm (0.76 mg/m^3)	0.057 ppm (0.38 mg/m^3)	0.039 ppm (0.26 mg/m^3)	0.021 ppm (0.14 mg/m^3)	0.015 ppm (0.10 mg/m^3)	Based on relative potency from GB[c]
GB	AEGL-1 (Nondisabling)	0.0012 ppm (0.0069 mg/m^3)	0.00068 ppm (0.0040 mg/m^3)	0.00048 ppm (0.0028 mg/m^3)	0.00024 ppm (0.0014 mg/m^3)	0.00017 ppm (0.0010 mg/m^3)	EC_{50} for miosis observed in adult female SD rats exposed to a range of GB vapor concentrations (0.01-0.48 mg/m^3) for 10, 60, and 240 min (Mioduszewski et al. 2002b)and miosis data from secondary and supportive studies with

Agent	Classification	10-min	30-min	1-h	4-h	8-h	End Point (Reference)
							marmosets (van Helden et al. 2001, 2002) and humans (Harvey 1952; and Johns 1952)
	AEGL-2 (Disabling)	0.015 ppm (0.087 mg/m^3)	0.0085 ppm (0.050 mg/m^3)	0.0060 ppm (0.035 mg/m^3)	0.0029 ppm (0.017 mg/m^3)	0.0022 ppm (0.013 mg/m^3)	Miosis, dyspnea, RBC-ChE inhibition, single fibre electro-myography (SFEMG) changes in human volunteers exposed at 0.5 mg/m^3 for 30 min (Baker and Sedgwick 1996)
	AEGL-3 (Lethal)	0.064 ppm (0.38 mg/m^3)	0.032 ppm (0.19 mg/m^3)	0.022 ppm (0.13 mg/m^3)	0.012 ppm (0.070 mg/m^3)	0.0087 ppm (0.051 mg/m^3)	Based on experimental SD rat lethality data (LC_{01} and LC_{50}); whole-body dynamic exposure to concentrations between 2 and 54 mg/m^3 for 3, 10, 30, 60, 90, 240, and 360 min (Mioduszewski et al. 2000, 2001, 2002a)
GD	AEGL-1 (Nondisabling)	0.00046 ppm (0.0035 mg/m^3)	0.00026 ppm (0.0020 mg/m^3)	0.00018 ppm (0.0014 mg/m^3)	0.000091 ppm (0.00070 mg/m^3)	0.000065 ppm (0.00050 mg/m^3)	Based on relative potency from GB[d]

AEGL-2 (Disabling)	0.0057 ppm (0.044 mg/m^3)	0.0033 ppm (0.025 mg/m^3)	0.0022 ppm (0.018 mg/m^3)	0.0012 ppm (0.0085 mg/m^3)	0.00085 ppm (0.0065 mg/m^3)	Based on relative potency from GB[d]
AEGL-3 (Lethal)	0.049 ppm (0.38 mg/m^3)	0.025 ppm (0.19 mg/m^3)	0.017 ppm (0.13 mg/m^3)	0.0091 ppm (0.070 mg/m^3)	0.0066 ppm (0.051 mg/m^3)	Based on relative potency from GB; supported by Wistar rat LC_{50}; dynamic chamber exposures at 21 mg/m^3 for three time periods of ≤30 min (Aas et al. 1985)[e]
AEGL-1 (Nondisabling)	0.00049 ppm (0.0035 mg/m^3)	0.00028 ppm (0.0020 mg/m^3)	0.00020 ppm (0.0014 mg/m^3)	0.00010 ppm (0.00070 mg/m^3)	0.000070 ppm (0.00050 mg/m^3)	Based on relative potency from GB[d]
AEGL-2 (Disabling)	0.0062 ppm (0.044 mg/m^3)	0.0035 ppm (0.025 mg/m^3)	0.0024 ppm (0.018 mg/m^3)	0.0013 ppm (0.0085 mg/m^3)	0.00091 ppm (0.0065 mg/m^3)	Based on relative potency from GB[d]
AEGL-3 (Lethal)	0.053 ppm (0.38 mg/m^3)	0.027 ppm (0.19 mg/m^3)	0.018 ppm (0.13 mg/m^3)	0.0098 ppm (0.070 mg/m^3)	0.0071 ppm (0.051 mg/m^3)	Based on relative potency from GB[e]

Agent	Classification	10-min	30-min	1-h	4-h	8-h	End Point (Reference)
VX[f]	AEGL-1 (Nondisabling)	0.000052 ppm (0.00057 mg/m^3)	0.000030 ppm (0.00033 mg/m^3)	0.000016 ppm (0.00017 mg/m^3)	0.0000091 ppm (0.00010 mg/m^3)	0.0000065 ppm (0.000071 mg/m^3)	Derived by relative potency from EC_{50} for miosis observed in adult female SD rats exposed to a range of GB vapor concentrations (0.01-0.48 mg/m^3) for 10, 60, and 240 min (Mioduszewski et al. 2002b) and miosis data from secondary and supportive studies of van Helden et al (2001, 2002), Harvey (1952), and Johns (1952) in marmosets and humans, respectively[g]
	AEGL-2 (Disabling)	0.00065 ppm (0.0072 mg/m^3)	0.00038 ppm (0.0042 mg/m^3)	0.00027 ppm (0.0029 mg/m^3)	0.00014 ppm (0.0015 mg/m^3)	0.000095 ppm (0.0010 mg/m^3)	Derived by relative potency from study of GB vapor exposure to exercising human volunteers exposed at 0.5 mg/m^3 for 30 min; miosis, dyspnea, inhibition of RBC-ChE, changes in single fibre electromyography (SFEMG) (Baker and Sedgwick 1996)[h]

AEGL-3 (Lethal)	0.0027 ppm (0.029 mg/m^3)	0.0014 ppm (0.015 mg/m^3)	0.00091 ppm (0.010 mg/m^3)	0.00048 ppm (0.0052 mg/m^3)	0.00035 ppm (0.0038 mg/m^3)	Derived by relative potency from experimental SD rat lethality data (LC_{01} and LC_{50}); whole-body dynamic exposure to GB vapor concentrations between 2 and 54 mg/m^3 for 3, 10, 30, 60, 90, 240, and 360 min (Mioduszewski et al. 2000, 2001, 2002a)[f]

[a]The derived AEGL values are for vapor exposures only. Percutaneous absorption of nerve agent vapors is known to be an effective route of exposure; nevertheless, percutaneous vapor concentrations needed to produce similar adverse effects are greater than inhalation vapor concentrations by several orders of magnitude. (For agent VX, the percutaneous vapor concentrations needed to produce similar adverse effects are greater than inhalation vapor concentrations by an approximate factor of 10.) Thus, the AEGL values presented are considered protective for both inhalation and percutaneous routes of exposure.

[b]Based on relative potency equal to that of agent GB (see Section 4.3 and Mioduszewski et al. [1998]).

[c]Agent GA is considered approximately one-half as potent as GB in lethality; thus, AEGL-3 values for GA are estimated by multiplying each time-specific AEGL-3 value for agent GB by a factor of 2 (see Section 4.3 and Mioduszewski et. al. [1998]).

[d]Agents GD and GF are considered approximately twice as potent as agents GA and GB for causing miosis, and they are equipotent to each other. Thus, AEGL-1 and AEGL-2 values are estimated by multiplying each time-specific AEGL-1 or AEGL-2 value for agent GB by a factor of 0.5 (see Section 4.3 and Mioduszewski et al. [1998]).

[e]Based on a relative potency for lethality of GD = GF = GB and lethality data of Aas et al. (1985) (which provides a 10-min AEGL-3 estimate of 0.27 mg/m^3 and a 30-min AEGL-3 value of 0.15 mg/m^3 and is thus supportive of the GD AEGL-3 estimate derived from relative potency) (see Section 4.3 and Appendix A).

[f]Based on relative potency. Agent VX is considered approximately 4 times more potent than agent GB (see Section 4.3.4, Grob and Harvey [1958], and Sidell and Groff [1974]).

[g]Derived from miosis effects noted in young adult female SD rats exposed to agent GB vapor at concentrations (0.010-0.48 mg/m^3) for 10, 60, and 240 min (Mioduszewski et al. 2002b). VX concentration to achieve same end point estimated by relative potency adjustment presented in footnote *f* above.

[h]Derived from transient effects noted in exercising human volunteers exposed to agent GB vapor at 0.5 $mg \cdot min/m^3$ for 30 min (Baker and Sedgwick 1996). VX concentration to achieve same end point estimated by relative potency adjustment presented in footnote *f* above.

[i]Derived from LC_{01} values for female Sprague-Dawley rats exposed to GB vapor in dynamic exposure chamber (Mioduszewski et al. 2000, 2001, 2002a). VX concentrations to achieve same end point estimated by relative potency adjustment presented in footnote *f* above.

The chemical-warfare agents discussed here are highly toxic organophosphate ester derivatives of phosphonic acid. They are commonly termed "nerve" agents as a consequence of their anticholinesterase properties and subsequent adverse effects on smooth and skeletal muscle function as well as the central nervous system. As a group, nerve agents are divided into the G-series agents ("G" for German, identifying these agents as among those secretly developed by the German Ministry of Defense before and during World War II—they contain a fluorine or cyanide substituent group) and the V agents (which contain a sulfur substituent group) (Sidell 1997). The G agents addressed in the current analysis include GA, or tabun (dimethylamidocyanoethylphosphate; $C_3H_{11}N_2O_2P$); GB, or sarin (isopropyl methylphosphonofluoridate; $C_4H_{10}FO_2P$); GD, or soman (pinacolyl methylphosphonofluoridate; $C_7H_{16}FO_2P$); and GF (O-cyclohexyl-methylfluorophosphonate; $C_7H_{14}FO_2P$). The V agent discussed in this document is VX (S-(diisopropyl aminoethyl) methyl phosphonothiolate, O-ethyl ester; $C_{11}H_{26}NO_2PS$). Agent VX is a persistent, "terrain denial" compound with a deliberately formulated low volatility; it is designed to contaminate surfaces.

Organophosphate (OP) nerve agents have been specifically designed and formulated to cause death, major injuries, or incapacitation to enemy forces in wartime. They are particularly effective in a military sense because of their potency. Detailed descriptions of nerve agent toxicity can be found in reviews by NRC (1999), Mioduszewski et al. (1998), Opresko et al. (1998), Sidell (1997), Munro et al. (1994), and Watson et al. (1989), among others.

Munitions containing agents GA, GB, and VX are stored at various military installations within the continental United States as part of the domestic unitary chemical warfare agent stockpile, which is undergoing congressionally mandated destruction (Carnes and Watson 1989). "Unitary" (as opposed to binary) munitions are those in which undiluted agents have been placed for immediate release upon firing or detonation.

According to information recently released by the Army at public meetings held in June 2001 in Pueblo, Colorado, the status of the project is as follows:

1. Disposal operations at Johnston Atoll were completed on November 29, 2000. Over one million munitions, containing over 2,030 tons of agents HD, GB, and VX, were destroyed.
2. As of June 6, 2001, the Tooele, Utah, facility had destroyed over 5,100 tons of agent GB, representing 37.4% of the original inventory at

Tooele. The Tooele facility began operations in August 1996. Demilitarization operations there are scheduled for completion in FY04.

3. Agents GB and/or GA are under secure storage and awaiting destruction at military facilities near Anniston, Alabama; Pine Bluff, Arkansas; Richmond, Kentucky; Tooele, Utah; and Umatilla, Oregon.
4. Agent VX is under secure storage and awaiting destruction at military facilities near Anniston, Alabama; Newport, Indiana; Pine Bluff, Arkansas; Richmond, Kentucky; Tooele, Utah; and Umatilla, Oregon.
5. The remaining demilitarization facilities are in various stages of construction.

Small quantities of agent GD are held in research and development facilities in the United States. Agents GA, GB, GD, and VX are listed as materiel thought to be located at some nonstockpile sites (DA 2001; USACMDA 1993a,b) and are being dealt with during installation restoration activities. The Chemical Weapons Convention (April 1997; Convention on the Prohibition of the Development, Production, Stockpiling and Use of the Chemical Weapons and on Their Destruction) has increased the interest in, and pace of, nonstockpile installation restoration.

Agent GF is believed to have been manufactured within Iraq during the Persian Gulf War (1990–1991) when precursors of agent GB (but not GF) were embargoed. Agent GF is currently considered of little strategic interest (Sidell 1997) but is included for completeness. With the possible exception of agent GF, all of the G agents identified above are considered potential military or terrorist threats.

Public and institutional concerns exist regarding potential agent release during unitary stockpile disposal, nonstockpile installation restoration activities, and potential chemical terrorism events (e.g., IOM 1999; Carnes 1989; NRC 1999; FEMA/DA 1996; DHHS 1988). A new dimension was added to consideration of this issue when it was determined that nerve agent GB had been used by a non-state terrorist group in two attacks on civilians in Japan during 1994 and 1995 (Sidell 1997; IOM, 1999). As a consequence, current domestic community emergency planning and preparedness often includes protocols for treating and managing exposure to chemical warfare agents (particularly nerve agents).

Experimental research specifically designed to improve the state of existing data sets quantifying toxic responses of mammals to nerve agent vapor exposure is currently underway and is supported by multiple military services. The AEGL analysis developed in this technical support document makes use of the most recent research findings (Mioduszewski et al. 2000, 2001, 2002a,b; van Helden et al. 2001, 2002; Anthony et al. 2002) from the

initiative. As the effort progresses, more of the assumptions necessary for developing AEGL estimates will be clarified. As new data and results become available in the next several years, assumptions will evolve. It is acknowledged that the current estimates represent a work in progress that will be updated as necessary.

Historical military approaches to chemical warfare (CW) agent protection and treatment of young and healthy soldiers are not necessarily suitable for application to heterogeneous civilian populations, and guidelines are needed for "safe and effective evacuation, decontamination, and other protective action" in the event of CW agent release in a civilian setting (IOM 1999). The development of AEGLs is intended to help address that need.

At present, the only CW agent control limits published in the United States for use in civilian community emergency preparedness planning are those developed by the Department of Health and Human Services (DHHS 1988; Thacker 1994). For the agents GA and GB, the current time-weighted average (TWA) applied as a no-adverse-health-effect level for 24-h continuous exposure to the general population is 3×10^{-6} mg/m^3. For the same agents, the 8-h TWA applied as a no-adverse-health-effect level for 8-h continuous workplace exposure for worker populations is 1×10^{-4} mg/m^3 (53 Fed. Reg. 8504 [1988]; DHHS 1988). Agents GD and GF, which are not part of the unitary stockpile, were not evaluated by DHHS in 1988. For VX, the TWA applied as a no-adverse-health-effect level for 24-h continuous exposure to the general population is 3×10^{-6} mg/m^3; the 8-h TWA applied as a no-adverse-health-effect level for 8-h continuous workplace exposure to worker populations is 1×10^{-5} mg/m^3 (DHHS 1988).

As part of a regularly scheduled review process, the Centers for Disease Control and Prevention (CDC) is currently reevaluating the 1988 agent control limits with application of recent risk assessment models and updated scientific data (67 Fed. Reg. 895 [2002]; DHHS 2002). The review is in progress (as of September 2002), and the CDC has not yet released a final position.

Acute Threshold Effects Levels developed by the CDC (Thacker 1994) are values of cumulative exposure (Ct) (concentration in mg/m^3 multiplied by time in minutes, or mg·min/m^3—Ct does not express the amount retained within the organism [Sidell 1997]). These cumulative exposure values are considered by the CDC to represent "lowest-observed-effect-levels" that "could be exceeded without danger" to the public and form the basis for planning protective actions, such as emergency evacuations, in the Chemical Stockpile Emergency Preparedness Program (CSEPP) of the Federal

Emergency Management Agency and the Department of the Army. The Acute Threshold Effect Levels are described by the CDC as protective of the general population (including consideration of vulnerable subgroups, such as infants, the elderly, and debilitated or ill persons) (Thacker 1994). The value for agent GB is 0.5 $mg \cdot min/m^3$, a protective cumulative exposure at which miosis is not expected to occur in humans (McNamara and Leitnaker 1971). If projected GB concentrations resulting from a release result in GB Cts >0.5 $mg \cdot min/m^3$, then the CDC considers protective measures (such as evacuation or shelter-in-place) warranted as a means of providing maximal protection to the general public. At the time of publication, the CDC has not established similar values for other G agents. The Acute Threshold Effects Level for agent VX is 0.4 $mg \cdot min/m^3$.

The database for toxicological effects in humans is more complete for agent GB than for the other G agents and for agent VX. Further, agent GB is the only G agent for which sufficient human data are available for use in deriving AEGL-1 and AEGL-2 estimates and the only G-agent for which sufficient laboratory animal data are available for deriving AEGL-1 and AEGL-3 values for all five AEGL time periods. In consequence, estimates for agents GA, GD, GF, and VX are, out of necessity, based on extrapolations of potency relative to the toxicity of agent GB.

Data for the derivation of AEGL-3 values for agent GB are from recent experimental studies of lethality in Sprague-Dawley rats (Mioduszewski et al. 2000, 2001, 2002a). AEGL-3 values for agent GD are derived from relative potency comparison with agent GB and limited inhalation lethality data for experimental exposures to Wistar rats (Aas et al. 1985).

All literature published in this technical support document is unclassified (i.e., not secret at any level, not confidential), including critical studies. Classified material relevant to AEGL assessment for these agents has been reviewed by document developers and has been found to contain no significant data that are not also found in unclassified reports. The technical support document itself was determined to be unclassified following examination by the Intelligence and Security Office of the U.S. Army Soldier and Biological Chemical Command (SBCCOM) (Aberdeen Proving Ground, Maryland) in July 2000.

Given the nature of the compounds under review, military literature is a major source of the relevant toxicity data. In consequence, some of the significant sources possess “limited distribution,” which is a separate issue from “classification.” Several sources possess a restricted distribution because of treaty restrictions on data access with allies, concerns regarding distribution of engineering information characterizing agent dissemination

or vapor generation contained in other sections of the same document, and related issues. To ensure public access to pertinent toxicity data originating from "limited distribution" materials, pertinent data from those sources have been incorporated into the technical support document. The technical support document itself was "cleared and approved for public access" by the Intelligence and Security Office of the U.S. Army SBCCOM (Aberdeen Proving Ground, Maryland) in July 2000. If additional details are desired, the U.S. Army Center for Health Promotion and Preventive Medicine will assist any request on a one-to-one basis. The point of contact is Ms. Veronique Hauschild (U.S. Army Center for Health Promotion and Preventive Medicine, Environmental Health Engineering, Bldg. E-1675, Aberdeen Proving Ground, MD 21010–5403).

All human exposure studies presented in this evaluation meet the criteria for acceptance for use in the AEGL process (e.g., there is evidence that subjects provided informed consent and that the studies were performed under appropriate clinical supervision) (NRC 2001).

The G agents are all viscous liquids of varying volatility (see Tables 1–2 through 1–5), with faint odors ("faintly fruity" or "spicy," odor of camphor) (DA 1990a,b; Dutreau et al. 1950; McGrath et al. 1953; MODa, unpublished material; all as cited in Marrs et al. 1996). However, these agents are considered odorless in field concentrations for all practical (military) purposes (DA 1990a,b). Odor thresholds are somewhat undefined (DA 1974, 1990a,b, 1992). Agent GA has been reported to have a faintly fruity odor, although it has no odor when pure (DA 1974, 1990a,b, 1992). For agent GB, the odor threshold was reported to be less than 1.5 mg/m^3 (DA 1974, 1990b, 1992; MODa, unpublished material, as cited in Marrs et al. 1996). For agent GD, the odor threshold was reported to be between approximately 1.5 mg/m^3 and 7.0 mg/m^3 (MODa, unpublished material, as cited in Marrs et al. 1996). Approximately 65% of adult subjects (N=34) exposed to GD at 3.3 to 7.0 mg/m^3 exhibited "mild nasal and airway symptoms" (Dutreau et al. 1950); a "median detectable concentration by odor for man is 7±2.4 mg/m^3." However, Dutreau et al. (1950) warn that it is doubtful that an untrained civilian could detect agent GD in sufficient time to avoid a partially incapacitating exposure. Agent GF is reported to have a sweet or musty odor of peaches and has an odor threshold between about 10.4 mg/m^3 and 14.8 mg/m^3 (McGrath et al. 1953, as cited in Marrs et al. 1996; DA 1990b).

As a class, G agents are more volatile and less persistent than the V agents; the vapor pressures and acute toxicity of the G-series agents are sufficiently high for the vapors to be rapidly lethal (USACHPPM 1996).

Within the G series, GB is considered a greater vapor hazard than agent GD (USACHPPM 1996). Agent GA represents a smaller vapor hazard and is expected to present a relevant contact hazard (USACHPPM 1996). The vapor pressure of agent GF is intermediate between that of agents GA and GD.

Agent VX is an amber-colored liquid with a molecular weight of 267.38; it has a vapor density of 9.2 (air=1) and a liquid density of 1.006 g/ml at 20 °C; its water solubility is 3 g per 100 g at 25 °C and 7.5 g per 100 g at 15 °C; and it has a low volatility (10.5 mg/m^3 at 25 °C) (DA 1990b). Agent VX is approximately 2,000 times less volatile than nerve agent GB (sarin) (DA 1990b). Because agent VX is considered odorless (Koon et al. 1959; DA 1990b), it possesses no olfactory warning properties.

Chemical and physical data for agents GA, GB, GD, GF, and VX are presented in Tables 1–2 through 1–6.

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

The acute lethal action of G agents and other anticholinesterase compounds results from their effects on the respiratory system at several levels: bronchoconstriction and excessive tracheobronchial secretion, paralysis of the diaphragm and other respiratory muscles, and depression of the CNS respiratory center (Mioduszewski et al. 1998).

G Agents

Based on extrapolations from historical animal data, the LCt_{50} for military personnel undergoing vapor exposures to GB has been estimated at 35 mg·min/m^3 for 2–10 min exposures at moderate temperatures (65–75 °F) for an individual with a respiratory minute volume of 15 liters (Reutter and Wade 1994). Reutter and Wade (1994) also estimated LCt_{50} values for military personnel undergoing vapor exposures to agents GA, GD, and GF; the estimates are 70 mg·min/m^3 for GA, 35 mg·min/m^3 for GD, and 35 mg·min/m^3 for GF. This Army report remains classified except for a summary table cited here that contains information on median exposure levels. The recommended LCt_{50} estimate for vapor exposure given in Reutter and Wade (1994) was calculated for 2-min exposure periods and then proposed

TABLE 1-2 Chemical and Physical Data for Nerve Agent GA

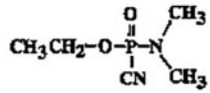
Parameter	Value	Reference
Chemical name	Dimethylamidocyanethylophosphate	Clark 1989; DA 1974, 1988, 1990a,b, 1992; Britton and Grant 1988; Small 1984; Windholz et al. 1983
Synonyms	Tabun; ethyl N,N-dimethyl phosphoro-amidocyanidate; N,N-dimethyl phosphoroamidocyanidate, ethyl ester.	
Chemical formula	$C_5H_{11}N_2O_2P$	
Chemical structure		DA 1990b
Molecular weight	162.13	DA 1990b
CAS Registry Number	77-81-6	DA 1974, 1990a,b, 1992
Physical state	Colorless to brown liquid	DA 1990b
Solubility in water (g/L)	98 (25 °C); 72 (20 °C)	DA 1990b
Vapor pressure (mm Hg, 20° C)	0.037	DA 1990b
Vapor density (air = 1)	5.63	DA 1990b
Liquid density (g/mL, 25° C)	1.073	DA 1990b
Melting point	-50 °C	DA 1974,1992
Boiling point	245 °C	DA 1974,1992
Flash point	78 °C	DA 1974,1992
Conversion factors in air	ppm = (0.15) × mg/m^3 (calculated) mg/m^3 = (6.6) × ppm (calculated)	Calculated from procedure outlined in ACGIH 2002 using molecular weight
logK_{ow}	1.18	Britton and Grant 1988
Bioconcentration factor (BCF)	Not available	
Henrys' law constant (atm m^3/mol)	1.52×10^{-7}	Opresko et al. 1998

TABLE 1-3 Chemical and Physical Data for Nerve Agent GB

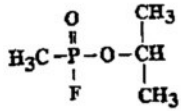
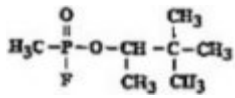
Parameter	Value	Reference
Chemical Name	Isopropyl methylphosphonofluoridate	Clark 1989; DA 1974, 1988, 1990a,b, 1992; Britton and Grant 1988; Small 1984; Windholz et al. 1983
Synonyms	Sarin; methyl phosphonofluoridate, isopropyl ester	
Chemical formula	$C_4H_{10}FO_2P$	
Chemical structure		DA 1990b
Molecular weight	140.10	DA 1990b
CAS Registry Number	107-44-8	DA 1974, 1990a,b, 1992
Physical state	Colorless liquid	DA 1990b
Solubility in water (g/L)	Miscible with water	DA 1990b
Vapor pressure (mm Hg at 20 °C)	2.10	DA 1990b
Vapor density (air = 1)	4.86	DA 1990b
Liquid density (g/mL, 20 °C)	1.102	DA 1990b
Melting point	-56 °C	Clark 1989; DA 1974, 1988, 1990a,b, 1992; Britton and Grant 1988; Small 1984; Windholz et al. 1983
Boiling point	158 °C	
Flash point	>138 °C	
Conversion factors in air	ppm = (0.17) × mg/m^3 (calculated) mg/m^3 = (5.7) × ppm (calculated)	Calculated from procedure outlined in ACGIH 2002 using molecular weight
logK_{ow}	0.15	Britton and Grant 1988
Bioconcentration factor (BCF)	Not available	
Henrys' law constant (atm m^3/mol)	5.34×10^{-7}	Clark 1989; DA 1974, 1988, 1990a,b, 1992; Britton and Grant 1988; Small 1984; Windholz et al. 1983

TABLE 1–4 Chemical and Physical Data for Nerve Agent GD

Parameter	Value	Reference
Chemical name	Pinacolyl methylphosphonofluoridate	Sidell 1997; Clark 1989; DA 1974, 1988, 1990a,b, 1992; Britton and Grant 1988; Small 1984; Windholz et al. 1983
Synonyms	Soman; phosphonofluoridic acid, methyl-1,2,2-trimethylpropyl ester	
Chemical formula	$C_7H_{16}FO_2P$	USACHPPM 1996
Chemical structure		DA 1990b
Molecular weight	182.178	DA 1990b
CAS Registry No.	96–64–0	USACHPPM 1996
Physical state	Colorless liquid	DA 1974
Solubility in water (g/L)	21 (20 °C)	DA 1990b
Vapor pressure (mm Hg at 25 °C)	0.40	Clark 1989; DA 1974, 1988, 1990a,b, 1992; Britton and Grant 1988; Small 1984; Windholz et al. 1983
Vapor density (air=1)	6.33	DA 1990b
Liquid density (g/mL, 25° C)	1.0222	DA 1990b
Melting point	−42 °C	DA 1990b
Boiling point	198 °C	DA 1990b
Flash point	121 °C	DA 1990b
Conversion factors in air	ppm=(0.13)×mg/m^3 (calculated) mg/m^3=(7.5)×ppm (calculated)	Calculated from procedure outlined in ACGIH 2002 using molecular weight
logK_{ow}	1.02	Britton and Grant 1988
Bioconcentration factor (BCF)	Not available	
Henrys' law constant (atm m^3/mol)	4.56×10^{-6}	Opresko et al. 1998

as a 2–10 min exposure estimate in the summary table. Thus, the LCt_{50} of 35 mg·min/m^3 assumes only short-term exposures of 2–10 min.

TABLE 1–5 Chemical and Physical Data for Nerve Agent GF

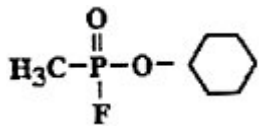
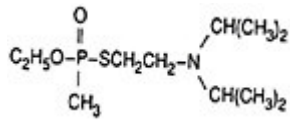
Parameter	Value	Reference
Chemical name	O-cyclohexyl-methylfluorophosphonate	DA 1990b
Synonyms	Cyclohexyl methylphosphonofluoridate (CMPF)	
Chemical formula	$C_7H_{14}FO_2P$	DA 1990b
Chemical structure		DA 1990b
Molecular weight	180.2	DA 1990b
CAS Registry Number	329–99–7	DA 1990b
Physical state	Liquid	DA 1990b
Solubility in water	0.37% (20 °C); almost entirely insoluble in water	DA 1990b
Vapor pressure (mm Hg, 25 °C)	0.044	DA 1990b
Vapor density (air =1)	6.2	DA 1990b
Liquid Density (g/mL, 20° C)	1.1327	DA 1990b
Melting point	−30 °C	DA 1990b
Boiling point	239 °C	DA 1990b
Flash point	94 °C	DA 1990b
Conversion factors in air	ppm=(0.14)×mg/m^3 (calculated) mg/m^3=(7.4)×ppm (calculated)	Calculated from procedure outlined in ACGIH 2002, using molecular weight
logK_{ow}	Not available	
Bioconcentration factor (BCF)	Not available	
Henrys' law constant (atm m^3/mol)	Not available	

TABLE 1–6 Chemical and Physical Data for Nerve Agent VX

Parameter	Value	Reference
Chemical name	O-ethyl-S-(diisopropylaminoethyl)methyl phosphonothiolate	Munro et al. 1999; DA 1990b
Synonyms	Agent VX; S-(2-diisopropylaminoethyl) O-ethyl methyl phosphonothiolate; ethyl-S-dimethylaminoethyl methylphosphonothiolate	
Chemical formula	$C_{11}H_{25}NO_2PS$	
Chemical structure		DA 1990b
Molecular weight	267.38	DA 1990b
CAS Registry Number	50782–69–9	DA 1990b
Physical state	Oily, amber-colored liquid	DA 1990b
Solubility in water (g/L)	3 g per 100 g at 25 °C 7.5 g per 100 g at 15 °C	DA 1974
Vapor pressure (mm Hg, 20° C)	0.0007 mm Hg at 20 °C	DA 1990b
Vapor density (air=1)	9.2	DA 1990b
Liquid density	1.006 g/cc at 20°C	DA 1990b
Melting point	−39 °C (calculated)	DA 1990b
Boiling point	298 °C	DA 1990b
Flash point	159 °C	DA 1990b
Conversion factors in air	$mg/m^3=(10.936)\times ppm$ $ppm=(0.0914)\times mg/m^3$	Calculated from procedure outlined in ACGIH 2002 using molecular weight
$\log K_{ow}$	Not available	
Bioconcentration factor (BCF)	Not available	

A subcommittee of the National Research Council's Committee on Toxicology (COT) has examined the Reutter and Wade (1994) analysis and recommends that the proposed LCt_{50} estimates for agents GA, GB, GD, and GF for estimating vapor inhalation and percutaneous exposure effects in exposed military populations "should be lowered" in light of the need for additional data characterizing vapor inhalation and percutaneous vapor toxicity. Furthermore, the subcommittee considered the estimates of Reutter and Wade (1994) inappropriate for civilian applications (NRC 1997).

Agent VX

From animal data, Reutter and Wade (1994) estimated a LCt_{50} for military personnel of 15 mg·min/m^3 for 2–10 min vapor exposures at moderate temperatures (65–75 °F) for an individual with a respiratory minute volume of 15 L. As in the case for agent GB, this LCt_{50} estimate was calculated for 2-min exposure periods and then proposed as a 2–10 min exposure estimate. Thus, the LCt_{50} for VX at 15 mg·min/m^3 assumes only short-term exposures of 2–10 min.

The subcommittee recommends that the Reutter and Wade (1994) proposed LCt_{50} estimate of 15 mg·min/m^3 for military personnel "should be lowered" because of the low to moderate degree of confidence in the estimation, which considered effects from vapor inhalation and percutaneous vapor exposures. Further, the subcommittee considered the estimates of Reutter and Wade (1994) inappropriate for civilian applications (NRC 1997).

Bide and Risk (2000) estimated the human 10-min LCt_{50} value for a VX aerosol based on lethality data for several animal species (see Section 3.1). The human LCt_{50} value was an estimated 7 mg·min/m^3 for a 70 kg man breathing 15 L/min for 10 min.

2.1.1. Case Reports

Agent GB

In 1994 and 1995, two incidents of chemical terrorism involving nerve agent GB (sarin) occurred in Japan; in both incidents, civilian populations were deliberately exposed to lethal concentrations by followers of a cult

originally local to Japan (Lillibridge 1995; Morita et al. 1995; Okumura et al. 1996; Sidell 1996). Because of the state of emergency at the time of release and the initial unknown nature of the source, exposures and dose-response could not be quantified.

The first incident occurred in June of 1994 in the central highland city of Matsumoto, Japan, where seven people died shortly after exposure to an unknown vapor later determined to be agent GB (Morita et al. 1995) released into a residential area during the night. The Matsumoto incident resulted in 56 hospital admissions as well as 253 cases in which the affected individuals sought medical consultation. Reports of "mild symptoms" were presented by eight out of 53 rescue personnel and one attending physician (Morita et al. 1995). Prompt deaths (N=3) and those who died before arriving at the hospital (N=4) appear to have been the result of respiratory insufficiency. At the time of the Morita et al. (1995) report, one patient remained "in a vegetative state because of anoxic encephalopathy"; a report on the outcome of that case has not yet been found.

The second occurrence, widely known as the Tokyo Subway Incident, took place on March 20, 1995. The same terrorist group responsible for the Matsumoto incident employed sources of passive, evaporative release of nerve agent GB in five individual subway cars serving three separate subway lines during morning commuter rush hours (Lillibridge 1995; Okumura et al. 1996; Sidell 1996). Of the 5,510 persons known to have been given medical attention, there were eight prompt deaths; four more died later (hours to days). The "later" group included individuals who had initially presented with "critical" respiratory effects requiring mechanical ventilation and intensive care (Lillibridge 1995). The 12 fatalities included commuters and subway transport employees, and death appeared to be the result of respiratory insufficiency. On hospital day 28, an additional death occurred as a consequence of "severe hypoxic brain damage" sustained during the release incident (Okumura et al. 1996). This delayed fatality was a previously healthy woman, 21 years of age, who presented without heartbeat or spontaneous respiration at the hospital but was revived with CPR and treated with agent antidotes. Plasma and RBC cholinesterase returned to normal within a period of days, but the patient eventually succumbed to hypoxic brain damage (Okumura et al. 1996).

Neuropathological examination of one individual who died 15 mo after being severely exposed to agent GB during the Tokyo subway terrorist attack indicated that the victim suffered marked nerve-fiber decrease in the sural nerve and moderate nerve-fiber loss in the sciatic nerve, with no changes in the dorsal root ganglion, dorsal roots, or posterior column of the

spinal cord (Himuro et al. 1998). The victim's CNS showed severe hypoxic-ischemic changes, which made it difficult to assess the specific effects of agent GB. Himuro et al. (1998) concluded that the observations were consistent with the "dying back" of the peripheral nervous system and might have been indicative of delayed neuropathy associated with inhibition of neuropathy target esterase (NTE). Himuro et al. (1998) cite as additional evidence of sarin-induced distal axonopathy an earlier study (Ishiyama 1996) in which degeneration of intramuscular nerve fascicles with preservation of the anterior horn cells was observed in a patient who died 1 mo after the subway attack.

2.2. Nonlethal Toxicity

Exposure to acutely toxic concentrations of nerve agents can result in excessive bronchial, salivary, ocular, and intestinal secretion, sweating, miosis, bronchospasm, intestinal hypermotility, bradycardia, muscle fasciculations, twitching, weakness, paralysis, loss of consciousness, convulsions, and depression of the central respiratory drive (Dunn and Sidell 1989). Minimal effects seen at very low exposure levels include miosis and rhinorrhea. The effects of exposures to very low concentrations of the nerve agents are evaluated in the literature, which includes clinical case reports as well as several studies using human volunteers. Key to acceptance of human subject data for use in the AEGL process is evidence that subjects provided informed consent and that the studies were performed under appropriate clinical supervision (NRC 2001). These criteria were met by the nonlethal studies summarized in Section 2.2.2.

A number of investigators consider *both* miosis and rhinorrhea to be early signs of exposure to cholinesterase inhibitors (Sidell 1997; Mioduszewski et al. 2002b; H.van Helden, Pulmonary and CNS Pharmacology Lab, TNO, the Netherlands, personal communtication; S.Tattersall, Biomedical Sciences Division, Porton Down, United Kingdom, personal communication). The presence of rhinorrhea can be indicative of inhalation exposure and/or development of systemic effects, while miosis in the absence of other signs or symptoms is a local effect to the pupillary muscles of the eye. In consequence, the presence of miosis is considered an appropriately sensitive indicator of direct vapor exposure and has the additional advantage of being readily recognized and quantifiable.

Recent nerve agent releases by terrorist groups have exposed civilian populations. Survivors of the incidents have been examined, and the resulting evaluations are summarized in Section 2.2.1.

2.2.1. Case Reports

Agent GB

Clinical case reports exist for the survivor population of the 1994 agent GB (sarin) release in Matsumoto, Japan, and the 1995 sarin release in Tokyo; no estimates of exposure concentrations could be found in the literature for either of these incidents. In the Matsumoto incident detailed above (see Section 2.1.1), Morita and his colleagues (Morita et al. 1995) published the clinical and laboratory findings of 264 people who sought treatment and the results of health examinations performed on 155 Matsumoto residents at 3 weeks (wk) postexposure. During initial treatment, severely poisoned individuals exhibited severe miosis, tachycardia followed by bradycardia, salivation, rhinorrhea, muscle fasciculations, and abnormal epileptiform EEGs. Other reported acute exposure signs and symptoms included headache, vision disturbances, fatigue, dizziness, nausea, dyspnea, ocular pain, and dysesthesia of the extremities. Clinical findings for the same group at the time of examination included decreases in serum cholinesterase, erythrocyte acetylcholinesterase, and serum triglycerides as well as serum potassium and chloride and increases in serum creatine kinase, leucocytes, and ketones in urine. For a period of up to 30 d following the incident, some of the severely exposed population exhibited slight continuous fever and some epileptiform EEG abnormalities (N=2 out of nine "severely affected people"). Nevertheless, follow-up examination revealed no persistent abnormal physical findings in any individual; acetylcholinesterase activity in erythrocytes and serum cholinesterase returned to normal within 3 mo in the examined population. Among some severe or moderately affected persons, subclinical miosis and some neuropathy were present 30 d after exposure. Morita et al. (1995) state that, in most people, "almost all symptoms of sarin exposure disappeared rapidly and left no sequelae."

In the hours following the Tokyo subway release of agent GB, the emergency department of St. Luke's International Hospital (located near

the affected subway stations) received 640 patients (Okumura et al. 1996). Additional details of the incident are provided above (see Section 2.1.1). Of the 640 admissions, 528 (82.5%) were diagnosed by Okumura and his colleagues (1996) as "mild" and exhibited "only eye signs or symptoms" such as miosis, eye pain, dim vision, and decreased visual acuity. Of the remaining 112 patients, one died in the emergency department, 107 were admitted as "moderate" cases (exhibiting "systemic signs and symptoms" such as weakness, fasciculations, convulsions, difficult breathing), and four were admitted as "severe" cases "requiring emergency respiratory support" such as intubation. Of the four severe cases, two patients experienced cardiac arrest but were revived, treated with agent antidotes and anticonvulsants, and eventually recovered fully (discharged on hospital day 3 and 5). Of the remaining two, both of whom required cardiopulmonary resuscitation, one recovered after vigorous treatment and was discharged on day 6. The remaining severely affected patient originally presented with no pulse and died on hospital day 28. For the three severe cases discharged, RBC-cholinesterase remained below normal activity levels for 51–72 d.

In the early 1970s, three men (ages 27, 50, and 52 y) working at Edgewood Arsenal (now Aberdeen Proving Ground in Edgewood, Maryland) in a chemical agent area containing stored containers of agent GB (sarin) were brought to an emergency room after sudden onset of rhinorrhea and respiratory discomfort approximately 20 min prior to arrival at the emergency room (Sidell 1974). It was determined later that one of the agent GB (sarin) containers in the work area had developed a leak and that the three individuals exhibiting signs had been working in the general area of the room where the leaking container was located. Examination indicated the presence of "mild respiratory distress, marked miosis with slight eye pain, rhinorrhea, a moderate increase in salivation, and scattered wheezes and rhonchi throughout all lung fields" (Sidell 1974). The men received no therapy but were observed for 6 h after emergency room arrival and were asymptomatic upon discharge except for eye irritation and "decreased vision in dim light." Blood cholinesterases were monitored and pupil diameter was recorded photographically for a period of 4 mo following exposure. Although 60–70% recovery of the ability to dark-adapt occurred within 2 wk, complete recovery of the ability to dark-adapt required 2 mo. Sidell (1974) did not report any estimates of the GB agent concentrations the men were exposed to.

Also in the early 1970s, a 52-y-old man in full protective gear employed in cleaning an agent GB-contaminated area at Edgewood Arsenal

(now Aberdeen Proving Ground in Edgewood, Maryland) experienced breathing difficulty and increased oral and nasal secretions (Sidell 1974). It was later determined that there was a crack in the man's voicemitter diaphragm through which exposure most likely had occurred. Upon arrival at the emergency room 5–10 min after the first symptom, he was convulsing and cyanotic. Other evident signs included labored breathing, muscular fasciculations, miosis, salivation, and rhinorrhea. He was treated aggressively with agent antidotes and provided assisted ventilation, and he recovered sufficiently to be able to walk through the ward by 9 h postadmission. Red blood cell cholinesterase (RBC-ChE) was monitored, as were EKGs. "While ChE activity in his blood was undetectable," the individual was conscious and alert (Sidell 1997). By 18 h postadmission, miosis was still evident. On day 4 and thereafter, the patient was asymptomatic; upon discharge 4 wk postexposure, he was "fully ambulatory and doing well." A 4-mo-postexposure EKG "was entirely within normal limit" (Sidell 1974). Sidell (1974) did not report any estimate of GB agent concentrations to which this individual was exposed.

In another incident of accidental exposure to GB vapors (0.09 mg/m^3 for an undefined duration resulting from a faulty ventilation hood), two men (ages 46 and 53 y) exhibited significantly lowered RBC-ChE for 80–90 d (one showed depression to 19% of baseline activity, the other to 84% of baseline activity) and extreme miosis that persisted for 30–45 d (Rengstorff 1985). These men exhibited no other signs or symptoms of nerve agent poisoning and required no treatment with antidotes.

2.2.2. Acute Studies

Agent GB

Vapor Exposures

Fairley and Mumford (1948) exposed 16 male volunteers to GB at 0.3 mg/m^3 for 0.5 min. Nine of the test subjects reported that they could detect the agent by smell; seven reported tightness of the chest, and 16 reported rhinorrhea.

McKee and Woolcott (1949) evaluated the effects of low concentrations of agent GB on 14 male volunteers. A single exposure to GB at 0.6 mg/m^3 for 1 min or at 0.06 mg/m^3 for 40 min resulted in miosis and slight tightness of the chest (4/4 subjects exhibited those signs and symptoms in

both the 1-min and 40-min tests; within 24 h, signs and symptoms resolved in subjects receiving 1-min exposures, although more than 48 h were required for resolution in subjects receiving 40-min exposures). Exposure of five individuals to GB at 0.06 mg/m^3 for 20 min/d resulted in miosis, but only after the fourth day of exposure. When the subjects were exposed to GB at 0.06 mg/m^3 for 40 min/d, miosis occurred on the first or second day and additional symptoms (headache, blurred vision, eye pain) appeared on the second, third, and fourth day of exposure.

In summarizing the toxicity studies conducted at Porton Down, United Kingdom, Mumford (1950) concluded that the threshold for ocular effects is 1.5–5.0 mg·min/m^3 (exposure times of 5–6 min) and that exposures to GB at 6–12 mg·min / m^3 (exposure times of 5–8 min) would result in moderate to severe discomfort due to miosis and frontal headaches.

In a study reported by Johns (1952) and Harvey (1952), 128 adult males volunteered to be exposed to GB concentrations ranging from 0.05 mg/m^3 to 3.0 mg/ m^3 for 2–20 min in a chamber. The corresponding Cts ranged from 1.0 mg·min/m^3 to 6.0 mg·min/m^3. The analytical methods used to measure the chamber concentrations of GB were not reported. Regression analysis of 150 observations, including 55 controls, indicated that the point at which a 50% decrease in pupil diameter would be attained was approximately 4.1 mg·min/m^3, with 90% confidence limits of about 2.7 and 5.7 mg·min/m^3 (Johns 1952). At the lowest test exposure level (0.05 mg/m^3 for 20 min), there were mean maximum decreases in pupil diameter of 0.82 mm (right eye) and 1.00 mm (left eye) (total of eight observations) compared with 0.36 mm (right eye) and 0.33 mm (left eye) in controls (55 observations). Johns (1952) defines “mild miosis” as a “decrease of 1 to 2 mm” in pupil diameter that usually disappears within 24 h. Although mild miosis, as defined by the author, was observed in some subjects at the lowest Ct tested (Ct=1.0 mg·min/m^3), other subjects exhibited mean maximal pupil decreases of <1 mm. This indicates that a likely response threshold was attained at this level of cumulative exposure. The results of the Johns (1952) study are presented in Table 1–7. It should be noted that untreated controls exhibited a pupil diameter decrease of ≥0.33 mm. Johns (1952) attributes this difference to observer bias and points out that there is still a relative difference between the control group and the exposure groups.

From the same overall study, Harvey (1952) reported signs and symptoms resulting from the GB exposures; those results are presented in Table 1–8.

TABLE 1–7 Decrease in Pupil Diameter (mm) Following GB Vapor Exposures

	Exposure Duration										
	20 min				4 min		2 min				
Concentration (mg/m^3)	0	0.05	0.2	0.3	1.0	1.3	0.5	1.0	2.0	2.3	3.0
Number of observations	55	8	11	11	12	4	15	9	8	7	10
Right eye; mean maximal decrease in pupil diameter (mm)	0.36	0.82	2.18	2.91	2.75	2.00	0.51	1.72	2.50	2.36	3.00
Left eye; mean maximal decrease in pupil diameter (mm)	0.33	1.00	2.18	3.00	2.59	2.22	0.60	1.67	2.92	2.07	3.00

Source: Johns 1952.

TABLE 1–8 Number of Test Subjects Showing Effects from GB Vapor Exposures

	Exposure Duration									
	20 min					2 min				
Concentration (mg/m^3)	0	0.05	0.1	0.2	0.3	0	0.5	1.0	2.0	3.0
Number of test subjects	4	14	34	11	12	4	15	9	15	10
Headache	1	2	1	1	8		4	1		4
Eye pain		2			6	1	3			6
Dimness of vision					7				4	7
Twitching of lids					2			2	2	
Rhinorrhea		3	20	11	12		2	9	15	10
Salivation					2					
Throat irritation					5		1		3	
Tightness in chest		1	12	2	9			6	11	4
Sweating					4					
Cramps		1			6				1	2
Nausea		1			3	1			1	
Vomiting					1				1	
Giddiness					5					
Concentration difficulty					8					
Malaise (“Grippe”)		2			6		1		7	7

Source: Harvey 1952.

In tests on human volunteers, Sim (1956) found that pupil constriction occurs more slowly and is less severe following exposure to GB at 5 mg/m^3 than at 10 or 15 mg/m^3 (1–3 min exposures). Some of the test subjects (number not given) reported restricted vision and eye pain.

Rubin et al. (1957) evaluated the effects of agent GB on the visual threshold of three adult volunteers. The test subjects were exposed to GB at 2 mg/m^3 for 2 min with the eyes protected or unprotected. With the eyes unprotected, the exposure resulted in moderate miosis and no other obvious signs of cholinesterase inhibition, but there was a significant elevation of the absolute visual threshold in the dark-adapted eye.

Oberst et al. (1968) conducted inhalation studies in which 125 volunteers were exposed to low concentrations of GB vapors in order to measure levels of GB retention and changes in RBC-ChE activity. In one series of tests in which resting subjects (N=90; minute volumes 5.6–8.4 L) were exposed to GB (concentrations in the supply chamber were 16.2 to 22.7 mg/m^3, average 20.7 mg/m^3) for 2 min, the calculated retained dose was 3.4–3.8 µg/kg and the percent inhibition of RBC-ChE activity was 39–63% (average 49%). In a second series of tests, in which exercising men (N= 35; minute volumes 41.5–64.9 L) were exposed to GB (supply chamber concentrations were 3.9 to 4.53 mg/m^3, average 4.19 mg/m^3) for 2 min, the calculated retained dose was 3.2–4.0 μg/kg and the percent inhibition of RBC-ChE activity was 29–58% (average 47%). The reported 2-min ChE_{50} dose for all 125 subjects (grouped data) was 3.95 μg/kg. From these data, the 2-min EC_{50} for cholinesterase inhibition can be estimated as approximately 21 mg/m^3 for resting men breathing about 7 L/min and about 4 mg/m^3 for exercising men breathing about 50 L/min. In these studies the subjects inhaled GB through a nosepiece or a mouthpiece; therefore, the potential effects of the agent on the eyes (i.e., miosis) could not be determined.

McNamara and Leitnaker (1971) applied mathematical and conceptual models to human and animal data and estimated that the threshold for neuromuscular effects and the ECt_{50} of GB for miosis in humans would be 4.0 mg·min/m^3 (0.2 mg/m^3 for 20 min). They further suggested that miosis would not occur at a Ct of 0.5 mg·min/m^3 (0.016 mg/m^3 for 30 min). McNamara and Leitnaker (1971) also estimated the Ct at which 50% inhibition of blood cholinesterase would occur; it was reported to be 20 mg·min/m^3 (0.67 mg/m^3 for 30 min). Blood cholinesterase activity was not expected to be affected at a Ct of 0.5 mg·min/m^3 (0.016 mg/m^3 for 30 min).

Callaway and Dirnhuber (1971) evaluated the "miotogenic potency" of GB vapor in humans exposed to GB "under goggles" (62 miosis responses

in 26 human volunteers). The "goggle" experiments were designed to deliver GB vapor directly to the air volume around the eye and enclose the vapor as a means of controlling the exposure (no inhalation or percutaneous exposure) and delivering the vapor directly to the surface of the eye (thereby reducing variability). An airstream of GB vapor (flow rate 0.1 L/min) was delivered to the space enclosed by each goggle. The unexposed pupil area of each eye was the baseline for pupil area decrement determinations for each eye. Exposure time periods ranged from 10 min to 5 h. Callaway and Dirnhuber (1971) reported a 50% loss of pupil area in the human dark-adapted eye at a Ct of 3.13 mg·min/m^3 (95% confidence interval [CI] =2.15–4.57 mg·min/m^3). A 90% loss of pupil area occurred at a Ct of 13.85 mg·min/m^3 (95% CI=6.00–32.02 mg·min/m^3).

Baker and Sedgwick (1996) exposed eight human volunteers to GB at 0.5 mg/m^3 for 30 min in an exposure chamber. During the exposure, test subjects walked at a rate of 96 paces per minute and breathed normally. It was reported that the test Ct of 15 mg·min/m^3 caused an inhibition of RBC-AChE activity to approximately 60% of individual baseline (reduction of 40%) at both 3 h and 3 d postexposure. Subjects exhibited miosis and, in some cases, photophobia and mild dyspnea following exposure. Respiratory symptoms resolved within minutes, and ocular effects resolved within 48 h. There were no clinical neuromuscular signs or symptoms; however, small changes in single fibre electromyography (SFEMG) of the forearm were measured at 3 h and 3 d postexposure and were still detectable at the first follow-up examination 4 to 15 mo postexposure. These changes were not detectable at the second follow-up examination 15 to 30 mo after exposure. Baker and Sedgwick (1996) suggested that these electrophysiological changes "may indicate subclinical onset of a non-depolarising type of neuromuscular block" that is fully reversible and has no clinical significance.

Oral Exposures

In clinical studies conducted by Grob and Harvey (1958), GB was administered orally in aqueous solution to eight normal subjects. Doses of 0.002 to 0.022 mg/kg resulted in 15–75% reduction in plasma and RBC-ChE activity. Grob and Harvey (1958) reported that the oral dose producing 50% depression of RBC-ChE was 0.01 mg/kg.

Intra-arterial Exposures

In clinical studies conducted by Grob and Harvey (1958), GB was administered by intra-arterial injection to eight normal subjects. Grob and Harvey (1958) reported that the intra-arterial dose of GB producing 50% depression of RBC-ChE was 0.003 mg/kg.

Agent GD

Fairley and Mumford (1948) exposed 15 male volunteers to GD at 0.3 mg/m^3 for 0.5 min. Fourteen men reported that they could detect the agent by smell, seven reported tightness in the chest, and 11 reported rhinorrhea.

Agent GA

Uhde and Moore (1945, as cited in Mioduszewski et al. 1998) reported that four men exposed to T2104 (agent GA) at a concentration of 0.35 mg/m^3 for 2 min were able to detect the agent by smell, and all reported slight, transient tightness of the chest, but none exhibited miosis. Ten men exposed to GA at 1.6 mg/m^3 for 2 min were able to detect the agent by smell, reported tightness of the chest, and exhibited miosis.

Agent VX

Local effects occurring at points of contact in the eyes and respiratory tract following exposure to low concentrations of VX vapor include miosis, rhinorrhea, and slight bronchoconstriction (Sidell 1992). These effects may occur without a significant decrease in activity of blood cholinesterases and without any signs of systemic toxicity (Sidell 1992). The ECt_{50} for mild effects (ocular effects, accompanied perhaps by chest tightness and rhinorrhea) resulting from vapor exposures has been estimated at 0.09 mg·min/m^3 for 2–10 min exposures at moderate temperatures (65–75 °F) for a respiratory minute volume of 15 L (Reutter and Wade 1994). Exposures sufficiently high to result in systemic uptake can result in muscular weakness, tremors, difficulty breathing, convulsions, paralysis, and death. The

ECt_{50} for severe effects resulting from vapor exposures has been estimated at 10 mg·min/m^3 for 2–10 min exposures at moderate temperatures (65–75 °F) for a respiratory minute volume of 15 L (Reutter and Wade 1994).

According to an unclassified NRC report (NRC 1997), the Reutter and Wade (1994) estimated ECt_{50} of 0.09 mg·min/m^3 for mild effects (ocular effects and rhinorrhea in humans) is based on the study by Bramwell et al. (1963) (percutaneous and direct ocular exposure to humans). The Bramwell et al. (1963) study is not considered credible for reasons that are discussed below under "Inhalation Exposures." This conclusion also is supported by the evaluation of a U.S. Surgeon General's review panel in an August 2000 public hearing (67 Fed. Reg. 894 [2002]; DHHS 2002).

Because agent VX is considered odorless, it possesses no olfactory warning properties.

Vapor Exposures

Sixteen volunteers participated in an odor detection study of stabilized and unstabilized VX (Koon et al. 1959). The agent was inhaled through an osmoscope attached to a chamber containing freshly generated agent vapor. The osmoscope permitted dilutions of the agent vapor with room air to yield concentrations down to one-sixty-fourth that in the chamber (0.05– 3.34 mg/m^3). Each subject sniffed the agent in the morning and in the afternoon on two successive days (presumably only one sniff at each time point). The estimated total doses for the four exposures ranged from 0.01 to 0.13 μg/kg. No significant changes in RBC- or plasma-ChE activity were demonstrated. Three subjects reported headaches the evening of the last test, and three other subjects reported slight chest tightness, dryness of the mouth, and nasal irritation for 30 min following the test. There was no agreement as to description of the odor. The median detectable concentration for VX vapor was estimated to be 3.6 mg/m^3 (95% CI=0.8–16.4 mg/m^3).

One of the few experimental attempts to evaluate human exposure to VX vapor for time durations greater than a few minutes is the historically important study of Bramwell et al. (1963) in which eight individuals were exposed for time periods ranging from 2.25 seconds (s) to 24 min to VX vapor concentrations ranging from 0.23 mg/m^3 to 5 mg/m^3 (Cts=0.7 to 25.6 mg·min/m^3). The Bramwell et al. (1963) study is not considered credible because of its seriously flawed exposure protocol but is presented here for completeness and context. The test subjects were exposed while stand

TABLE 1–9 ChE Inhibition in Humans Following Exposure to VX Vapors

		Exposure Conditions			Max ChE Inhibition (% depression)
Trial	Subject	Time (min)	Concentration (mg/m^3)	Ct (mg·min/m^3)	
R1	SHE	3	0.2	0.6	20
R2	BIS	3	0.35	0.9	18
R3	LAD	3	0.31	0.9	22
R4	BUR	3	0.37	1.1	17
R5	BRA	3	0.4	1.2	14
R6	HOP	3	0.48	1.4	10
R7	CRO	3	0.57	1.7	12
R8	SHE	1.5	1.6	2.4	26
R9	BRA	1.5	1.73	2.6	25
R10	BUR	1.5	1.73	2.6	21
R11	BIS	1.5	1.93	2.8	28
R12	LAD	1.5	2.0	3.0	41
R13	HOP	1.5	2.07	3.1	18
R14	HOL	1.5	2.07	3.1	28
R15	CRO	1.5	2.4	3.6	20
R16	CRO	6	0.8	4.8	44
R17	LAD	7	0.79	5.5	70

		Exposure Conditions			Max ChE Inhibition (% depression)
Trial	Subject	Time (min)	Concentration (mg/m^3)	Ct (mg·min/m^3)	
R18	SHE	6	1.02	6.1	47
R19	BUR	6	1.06	6.4	46

Note: These data are not considered credible for use in deriving AEGLs (see text).
Source: Bramwell et al. 1963, as cited in Reutter et al. 2000.

ing or seated at the mouth of a tunnel from which VX vapor was flowing in an airstream at 1 m/min at a temperature of 32 °C. Only the head and neck of the test subjects were exposed. A total of 19 exposures were conducted without respiratory protection (see Table 1–9). All but one of the tests were conducted with eyes closed without the use of eye protection in the form of goggles or face mask. The only symptoms noted during the exposures were slight tightness in the throat and upper respiratory tract; these symptoms were not reported by all subjects. In the individual exposed with eyes open (0.31 mg/m^3 for 3 min), miosis developed suddenly 20 to 30 min postexposure and was maximal at 1.5 h postexposure. In the individuals exposed with eyes closed, some miosis usually developed 1 to 3 h postexposure. The degree of miosis was quite variable among the individuals and appeared to be concentration-dependent. The miosis was often accompanied by a fluttering or twitching of the eyelids. Although the muscle effects were clearly reported by the subjects, they were not always obvious to the observers. Rhinorrhea occurred within 30 min of exposure in 14 of 19 trials. In four other trials, it developed more slowly; in one, it did not develop at all. Excessive salivation, lasting for about an hour, was reported in one subject after a 6-min exposure to a concentration of 1.06 mg/m^3. Two hours postexposure, one individual experienced some nausea and sweating; RBC-ChE activity was 60% depressed at that time. These effects abated somewhat and then recurred later when ChE inhibition had reached 70%. Several individuals also experienced malaise and lethargy. Based on all 19 trials, the inhaled dose estimated to produce inhibition of 50% of the RBC-ChE activity (ChE_{50}) was 13 μg/kg. However, the authors thought that apprehension had increased the subjects' minute volume during initial exposures. That would have effectively increased the dose to which the individuals were exposed and was thought to account for a relatively shallow probit slope. When those data were excluded, the estimated ChE_{50} was about 8 μg/kg, which was thought to compare favorably with intravenous data.

The Bramwell et al. (1963) study is not considered credible for use in deriving AEGLs for agent VX. Reutter et al. (2000) examined the Bramwell et al. study as a potential critical study for the estimation of worker population levels (WPLs) and general population levels (GPLs) for chronic exposure to VX vapor (8-h time-weighted average for WPL; 24-h continuous exposure for GPL). Reutter et al. (2000) rejected the Bramwell et al. study because of multiple deficiencies; the concentration of VX to which the subjects were exposed could not be determined (subjects were seated in front of a "tunnel" down which generated VX vapor flowed in an

airstream of known velocity), both *C* and *t* were varied (resulting in no replicate cumulative exposures), and the organic sol vent benzene was used to help disperse the agent in the airstream to which subjects were exposed (Bramwell et al. did not address the potential effect of the carrier solvent on agent absorption by the subject). The majority of a U.S. Surgeon General's review panel concurred with the appraisal of Bramwell et al. (1963) at a public hearing convened by the CDC to examine the Reutter et al. (2000) report (67 Fed. Reg. 894 [2002]; DHHS 2002).

Oral Exposures

In clinical studies conducted by Sidell and Groff (1974), single oral doses of VX at 2–4.5 μg/kg (stock solution in absolute ethanol diluted in a solution of saline and dextrose and swallowed by each subject under supervision) produced gastrointestinal symptoms in 5 of 32 test subjects (more specific dose-response data not reported). Regression analysis of the dose-response data indicated that the RBC-ChE_{50} was 2.3 μg/kg. Sidell and Groff (1974) reported that the oral dose of VX needed to produce 70% ChE inhibition (4 µg/kg) was 3 times greater than that needed to produce the same effect after intravenous administration.

Sim et al. (1964) reported no signs of toxicity in seven human volunteers receiving VX at 1.43 μg/kg/d for 7 d (in four daily doses of 500 mL drinking water); however, average RBC-ChE activity was reduced 60% (to 40% of baseline values). The Sim et al. (1964) study resulted in a lower RBC-ChE_{50} value than the Sidell and Groff (1974) oral study, probably because of the cumulative effects of VX given over the 7 d in the Sim et al. study. The total dose in the Sim et al. (1964) study was about twice that used in the Sidell and Groff (1974) oral study.

Intravenous Exposures

Several studies have been conducted in which human volunteers were injected intravenously with VX. The experiment of Kimura et al. (1960) was performed with the informed consent of the participants, under full clinical supervision and in a hospital setting considered suitable at the time (resuscitation team at bedside "to administer atropine, oximes, oxygen, artificial resuscitation, and tracheotomy if indicated"). Kimura et al. (1960) reported that a 30-s intravenous injection of 0.04 μg/kg in one adult test

subject caused frontal and retrobulbar headaches starting 45 min after the injection. The subject reported being tired and appeared irritable to observers, but no change in RBC or whole blood cholinesterase activity was observed. A subsequent 30-s intravenous injection of 0.08 μg/kg 3.5 h later resulted in a 2-fold increase in airway resistance, a 25–30% decrease in respiratory rate, and a 15% drop in pulse rate 15 min after the exposure, but no change in RBC-ChE. Headaches began 20 min postexposure, and minute volume increased from 15 L to 32 L 30–45 min postexposure. Peak effects (increased sweating, lightheadedness, and abdominal cramping) appeared about 45 min after the dose was administered. A single 30-s intravenous dose of 0.225 μg/kg in one test subject resulted in a 27% decrease in baseline RBC-ChE activity within 15 min as well as retrobulbar headaches. Many of these observed effects are for the single subject participating in the dose-response range-finding study—Dr. Van Sim, MD, a principal investigator of the reported study. Six additional subjects (volunteers identified by subject code) received VX at 1 μg/kg by intravenous infusion over 1.75 to 4 h periods and exhibited 50–60% depression in cholinesterase activity but no signs of toxicity (except for one 84-kg individual who reported headaches).

The Kimura et al. (1960) study meets the criteria for acceptance of human subject data for use by the AEGL process (e.g., evidence that subjects provided informed consent and that the studies were performed under appropriate clinical supervision).

In clinical studies conducted by Sidell and Groff (1974), 34 test subjects were given VX by intravenous injection. The administered dose ranged from 1.2 to 1.7 μg/kg. An intrvenous dose of 1.5 μg/kg administered to 18 test subjects resulted in dizziness, nausea, and vomiting in 11, 4, and 6 individuals, respectively; RBC-ChE was depressed 55–90% from baseline values (average about 75%). The test subjects exhibited a significant decrement in performance on a number facility test within 1 h after treatment. Regression analysis of the dose response data indicated that the RBC-ChE_{50} was 1.1 μg/kg (three individuals tested at 1.2 μg/kg, 1.3 μg/kg, 1.4 μg/kg, and 1.7 μg/kg; four at 1.6 μg/kg; and 18 at 1.5 μg/kg; estimated from graphic presentation of the data) (Sidell and Groff 1974).

Percutaneous Exposures

Dermal vapor absorption is a low priority for this compound, although there are certain release events that generate a dermal vapor threat. It is

generally acknowledged that a specific toxicological end point for vapor exposure to nerve agent VX would be achieved at a lower concentration exposure for the inhalation route than for other routes (e.g., the estimated human LCt_{50} for percutaneous vapor exposure to agent VX is 150 mg·min/m^3, while the estimated human LCt_{50} for inhalation vapor exposure to agent VX is <15 mg·min/m^3) (NRC 1997). Thus, AEGL estimates based on inhalation exposures are considered protective for both inhalation and dermal routes.

In studies conducted by Bramwell et al. (1963, as cited in Reutter et al. 2000) eight individuals were exposed for time periods ranging from 2.25 s to 24 min to VX vapor concentrations ranging from 0.23 mg/m^3 to 5 mg/m^3 (Cts=0.7 to 25.6 mg·min/m^3). The test subjects were exposed while standing or seated at the mouth of a tunnel from which VX vapor was flowing in an airstream at 1 m/min at a temperature of 32 °C. Only the head and neck of the test subjects were exposed. Thirty-five of the exposures were performed with eyes closed (but without the use of eye protection in the form of goggles or face mask) and with respiratory protection (a nose clip was used and the subjects were breathing through a spirometer connected to a respirator canister). ChE inhibition was measurable within an hour of exposure and was greatest at 8–12 h postexposure. No signs or symptoms were noted during the exposure periods; however, 30 min or more after the initial exposure, miosis appeared in nearly all subjects and became maximal several hours later. It was usually accompanied or followed by fluttering and twitching of the eyelids and was more pronounced at the higher concentrations. Flushing of the skin of the head and neck was observed in five of the eight subjects, and all eight individuals reported local sweating in one or more tests. Although some subjects had the perception that they were experiencing "tunnel vision" postexposure, visual perimetry studies following three of the exposures were not confirmatory. Nor were there any changes in visual acuity or color vision. Five hours postexposure, one subject developed flatulence and abdominal discomfort. An hour later he did not feel well and was experiencing waves of nausea. Eight hours postexposure, he deteriorated rapidly and experienced severe nausea and vomiting. At that time, his RBC-ChE activity was only 30% of baseline; no further inhibition occurred. Bouts of vomiting and malaise continued, and he experienced cold sweating, pallor, and a feeling of motion sickness—minus the vertigo. At 12 h postexposure, he was able to sleep, but experienced a nightmare shortly after falling asleep. By the next morning no signs or symptoms remained. The Bramwell et al. (1963) study is not considered credible for deriving AEGL values.

Lubash and Clark (1960) reported that percutaneous doses of undiluted VX (20 μg/kg or 35 µg/kg) applied to the volar forearm of male volunteers resulted in significant decreases in blood ChE as well as signs and symptoms of toxicity (lightheadedness, nausea, vomiting, diarrhea, hyperactive bowel sounds, epigastric discomfort, insomnia, and nightmares) in two of four subjects dosed with 20 μg/kg and in two of four subjects dosed with 35 μg/kg (eight total subjects).

Sim (1962) reported that head and neck areas were the most sensitive to percutaneously applied VX. A dose of VX at 5 μg/kg applied to these areas resulted in signs and symptoms of systemic toxicity (nausea, vomiting, and weakness) in 54% (28 of 40) of the tested individuals. Whole blood ChE was 50% of normal in 5.8 h and 33.5% of normal in 8.5 h. It was estimated that a VX dose of 5.1 μg/kg would be necessary to result in RBC-ChE_{30} (this end point was chosen because median ChE depression of 30% was associated with the onset of gastrointestinal signs and symptoms of nausea and vomiting).

Cresthull et al. (1963) studied the effects of percutaneous absorption of VX vapors on whole blood ChE activity in 29 male volunteers. Exposures were to the arm or forearm. The VX concentrations ranged from 1.2 to 12.2 mg/m^3 and the exposure times were from 2 to 75 min (Cts ranged from 6 to 765 mg·min/m^3). Two men were exposed at 1.2–1.5 mg/m^3 for 5 min (500 cm^2 surface area exposed); six to 2.5–4.9 mg/m^3 for 5–10 min (500 cm^2 surface area exposed); four to 4.8–7.3 mg/m^3 for 12–20 min (500 cm^2 surface area exposed); ten to 4.5–8.0 mg/m^3 for 20–60 min (1,000 cm^2 surface area exposed); and seven to 8.5–12.2 mg/m^3 for 60–75 min (1,000 cm^2 surface area exposed). The median decrease in whole blood ChE in those groups was 5%, 3%, 8%, 18%, and 43%, respectively. Although whole blood ChE was inhibited as much as 76% at 20 h after exposure, none of the test subjects exhibited any toxic signs. Cresthull et al. (1963) estimated that the whole blood ChE_{50} vapor concentration for percutaneous exposures would be 141 mg·min/m^3. The value was reported to be not statistically meaningful because of the wide confidence limits (lower 95% CI=35 mg/m^3); however, by comparison with data for exposures to VX aerosols, Cresthull et al. concluded that the estimated ChE_{50} of 141 mg·min/m^3 was acceptable. Cresthull et al. (1963) also estimated that 1 to 1.25 h whole-body percutaneous exposure to a Ct of 38 mg·min/m^3 (0.51 mg/m^3 for 75 min) would not cause any signs of toxicity other than "partial" lowering of whole blood ChE (activity inhibition between 0% and 31% from baseline).

Bowers et al. (1964) evaluated behavioral changes in 93 volunteers who were exposed percutaneously to small amounts of liquid EA-1701 (agent VX). The actual amounts of VX applied were not reported. The test subjects were divided into three postexposure groups depending on the level of reduction in their whole blood ChE (there was no control group). Of 32 individuals whose whole blood ChE was 81–100% of control values (not explicitly stated but presumed to be individual preexposure values) following exposure, 6% showed symptomatology of intellectual impairment (impairment of ability to perform simple arithmetic tasks, inability to perform serial sevens, impairment of performance in reading or standard games of concentration, and other subjective symptoms such as "impairment in orientation"), and 3% reported unusual dreams. Of the 24 whose whole blood ChE was 40–80% of control values, 4% showed symptomatology of intellectual impairment (by the measures reported above), 33% reported unusual dreams, 8% exhibited anxiety (determined by the appearance of palpitations coupled with other, subjective symptoms such as "restlessness"), and 4% exhibited psychomotor depression (determined by the appearance of reply latency, slowed speech, and evidence of fatigue in addition to other, subjective symptoms such as reported feelings of being "slowed down"). Of the 37 whose ChE was 10–40% of control values, 57% showed symptomatology of intellectual impairment (by the measures reported above), 38% reported unusual dreams, 30% exhibited anxiety, and 57% exhibited psychomotor depression. The more severely affected cases exhibited mood alterations as determined by Clyde mood card sort before and after exposure, and some developed nausea and vomiting. Miosis, bronchoconstriction, hypermotility of the lower bowel, and muscle fasciculations were not observed in any of the test subjects. Bowers et al. (1964) concluded that, with the exception of excessive dreaming, psychological symptomatology did not develop in the exposed individuals unless whole blood ChE fell to 40% or less of control values. Very few of the test subjects whose blood ChE was 80% or more of control values exhibited any signs.

Data compiled by Sidell (1992) revealed that, for individuals exposed to VX percutaneously, gastrointestinal signs (vomiting) occurred in 0.6% (1/166) when RBC-ChE activity was at 50% of control values and in 8% (2/24) when RBC-ChE levels were 40–49% of controls. Thirty-three percent exhibited such signs when RBC-ChE levels were 30–39% of controls, and 45% (19/42) exhibited signs when RBC-ChE levels were 20–29% of

controls. Sixty-seven percent (16/24) exhibited effects when RBC-ChE levels fell to less than 20% of control values.

2.2.3. Epidemiologic Studies

There are no human epidemiologic studies with dose-response data suitable for deriving AEGL estimates for the G agents.

Occupational exposures to agent GB have been associated with altered electroencephalograms (EEGs) (Duffy et al. 1979; Burchfiel and Duffy 1982). Burchfiel and Duffy (1982) evaluated the wake and sleep EEGs of 77 industrial workers who had been exposed at least once to agent GB (sarin); however, no exposures had occurred in the year preceding the study. Spectral analysis of the EEGs indicated significant increases in brain beta activity (12–30 Hz) in the exposed group compared with nonexposed controls, and sleep EEGs indicated significantly increased rapid eye movement in the exposed workers. Combinations of EEG components were subjected to computer analysis in an attempt to identify an exposed individual by EEG characteristics; however, the results were inconclusive. Burchfiel and Duffy (1982) concluded that there might be a threshold for this type of effect. In evaluating the data of Burchfiel and Duffy (1982), DHHS (1988) considered the EEG changes to be "of questionable significance—given the difficulty of demonstrating such changes and the absence of clinically significant effects even when EEG changes are present."

A retrospective analysis of possible chronic or delayed adverse health effects among servicemen who participated in chemical agent effects and therapy testing at Edgewood Arsenal during the years 1955–1975 was conducted by the Committee on Toxicology of the National Research Council (NRC 1985). The primary source of information was provided by participant response to a questionnaire, but there were no exposure data from which to derive a dose-response relationship. The chronic health effects of concern were "excess cancer risk, and adverse mental, neurologic, hepatic and reproductive effects."

Evaluation of questionnaire response indicated that data provided by subjects historically tested with anticholinesterase compounds did not significantly differ from that of control subjects or those tested with other compounds when self-evaluations of current health status were compared. The report candidly pointed out that the experimental design and comparison groups available were such that "only large effects were likely to be

uncovered" because of the resulting large standard errors, self-reporting, and the potential for more than one exposure to eventually result in development of the same biological end point (NRC 1985).

A number of studies have been conducted on individuals exposed to agent GB as a result of terrorist attacks in Japan. Morita et al. (1995) reported clinical findings for several hundred people who were exposed to agent GB in the city of Matsumoto in 1994. Subclinical miosis and neuropathy were still present in some individuals 30 d after exposure; however, most individuals exhibited no clinical signs of toxicity 6 mo after the exposure.

Several follow-up studies have examined the health of victims of the Tokyo subway terrorist attack that occurred in March of 1995. No clinical abnormalities were detected in 640 patients examined 3 mo after the incident (Okumura et al. 1996). Kato and Hamanaka (1996) examined 96 victims for ocular effects. The primary ocular signs and symptoms included miosis, conjunctival injection, and ocular pain. Some individuals had temporary blurring of vision, 36 patients complained of subjective accommodation impairment, and in 30 patients there were indications that agent GB (sarin) had caused a reduction in intraocular pressure (intraocular pressure was 11.6±1.9 mm Hg within 2 h of exposure but increased to 14.6± 1.8 mm Hg when the pupil diameter returned to normal). These signs and symptoms spontaneously resolved within 3–21 d after exposure in most cases. Kato and Hamanaka (1996) note that none of the victims developed corneal injury, glaucoma attack, or retinal detachment, and although the ocular condition of the patients returned to normal, they suggest that exposure to agent GB may increase the risk of angle-closure glaucoma caused by anterior shift of the lens, retinal detachment, and vitreous hemorrhage caused by extensive contraction of the ciliary muscles. Murata et al. (1997) evaluated neurophysiological deficits in 18 victims of the subway attack who had exhibited signs and symptoms of agent GB poisoning (i.e., headache, miosis, increased lacrimation, dyspnea, nausea, diarrhea, paraesthesia, and decreased serum ChE activity). It was reported that 6 mo after the exposure, the exposed but no longer symptomatic individuals exhibited significantly prolonged latencies in event-evoked potentials and visual evoked potentials suggestive of persistent cognitive and visual dysfunction.

In another study, Yokoyama et al. (1998a,b) evaluated chronic neurobehavioral effects in nine male and nine female patients 6–8 mo after the incident. Although this study is for a very small number of those affected

(only 18 out of approximately 5,500 people) and suffers from low recruitment, the results will be presented here for completeness. The neurobehavioral tests included (1) digit symbol (psychomotor performance), (2) picture completion (visual perception), (3) digit span (attention and memory), (4) finger tapping (psychomotor performance), (5) reaction time (psychomotor performance), (6) continuous performance test (sustained visual attention), (7) paired-associate learning (learning and memory), (8) General Health Questionnaire (GHQ) (psychiatric symptoms), and (9) Profile of Mood States (POMS) (mood). Fifteen controls were used in the tests. Analysis of covariance of the test results suggested to the investigators that "perhaps a chronic effect on psychomotor performance [digit symbol test only] was caused directly by acute agent GB (sarin) poisoning; on the other hand, the effects of psychiatric symptoms (GHQ) and fatigue (POMS) appeared to result from post-traumatic stress disorder induced by exposure to sarin." Yokoyama et al. (1998c) have also reported vestibulocerebellar effects (increased postural sway) in 18 patients tested 6–8 mo after the incident. Postural sway was significantly greater than controls in exposed females but not in males. In both genders postural sway was correlated with the plasma cholinesterase activity measured immediately after the exposure.

The U.S. Department of Defense reported in 1997 that military personnel might have been exposed to nerve agents as a result of the demolition of Iraqi munition storage sites. Retrospective studies have evaluated the post-war health of soldiers who may have been exposed to nerve agents. Landrigan (1997) reviewed the principal epidemiologic studies published before 1997. Kang and Bullman (1996) reported a 0.8% higher death rate among Gulf War veterans (10.4%) compared with other veterans of the same time period (9.6%); this difference was largely due to accidents, and no excess deaths from suicides or specific diseases were observed. Gray et al. (1996, 1999) reported no consistent pattern for increased occurrence of any specific disease or hospitalization among Gulf War veterans. Further, Gray et al. (1999) indicate that "this data analysis does not support the hypothesis that Gulf War veterans are suffering postwar morbidity from subclinical nerve agent exposure."

Other epidemiologic studies have reported an increase in neurologic disorders among selected groups of Gulf War veterans but have not linked any reported signs, symptoms, or clinical effects with potential nerve agent exposure (Goldstein et al. 1996; Kotler-Cope et al. 1996; Haley et al. 1997a,b; Haley and Kurt 1997; Hom et al. 1997; Schwartz et al. 1997; Vasterling et al. 1998).

Epidemiologic studies regarding human exposure to agent VX were not found in the available literature.

2.3. Neurotoxicity

The G agents (GA [tabun], GB [sarin], GD [soman], and GF) and agent VX are toxic organophosphate ester derivatives of phosphonic acid. They are commonly termed "nerve" agents as a consequence of their potent anticholinesterase properties and subsequent adverse effects on both smooth and skeletal muscle function as well as the central and peripheral nervous systems. These neurotoxic properties were discussed in detail in Sections 2.1 and 2.2.

Although the inhibition of cholinesterases within neuroeffector junctions or the effector itself is thought to be responsible for the major toxic effects of nerve agents, these compounds can affect nerve impulse transmission by more direct processes as well (e.g., direct effects on neurotransmitter receptors) (see Section 4.2).

2.4. Developmental and Reproductive Toxicity

The retrospective study of agent-exposed servicemen discussed in preceding sections (NRC 1985) requested self-reported information on fertility. Two comparison groups of men were used. One was a "no chemical test" (NCT) group who met the requirements for military service but did not meet the more rigorous requirements (physical and mental screening exams for contraindications) necessary for chemical exposure tests. Those individuals were exposed to placebos, equipment only, or "FDA approved drugs" not otherwise identified. A second comparison group comprised men tested with compounds other than those being evaluated in a particular test, the "other chemical test" (OCT) group. These individuals also met the requirements for military service. They were exposed to test chemicals other than the chemicals of interest. The OCT compounds appear to include cannabinoids, "approved drugs," and "innocuous chemicals and controls" not otherwise identified (NRC 1985). When the collected data were adjusted for volunteer age when the last test was performed (to accommodate national trends toward smaller and delayed families), there "was no difference between the observed fertility pattern of men exposed to anticholinergic chemicals and that expected on the basis of men who were

exposed to other chemicals" (NRC 1985). Nevertheless, these data are not useful for application to the derivation of an AEGL given that no exposure data were collected.

Iranian soldiers and civilians were exposed to multiple chemical warfare agents during the Iran/Iraq conflict. Exposures may have been to the nerve agents GA and/or GB as well as to the vesicant sulfur mustard. Follow-up studies have been conducted on some of the individuals. It has been reported that the offspring of these chemical warfare victims born after the Iran/Iraq conflict were more likely to have birth defects than those born before the war (Pour-Jafari 1994a). It was also reported that the off-spring had an altered gender ratio (Pour-Jafari 1994b). Because of the possibility that exposures to multiple chemicals had occurred, it is impossible to determine if, or to what extent, exposure to any of the G agents contributed to the reported effects.

There have been several reports of potential increased incidence of birth defects among the offspring of military personnel who served in the Persian Gulf War. Araneta et al. (1997) reported a slight increase (relative risk 3.03, with 95% CI=0.63–20.57) in Goldenhar syndrome among infants born in military hospitals to Gulf War veterans. Goldenhar syndrome is a craniofacial anomaly of unclear etiology. According to Araneta et al. (1997), suggested associations with its occurrence have included chromosomal abnormalities, a genetic pattern of inheritance, maternal diabetes, or prenatal exposure to several controlled or therapeutic drugs (e.g., cocaine, tamoxifen); the role of male-mediated effects is undefined. At least one mother of one case infant exhibited mild facial asymmetry upon examination, and the family of another case infant had a history of birth defects. In all five cases of confirmed Goldenhar syndrome among the 34,069 infants born to veterans of the Gulf War and included in this retrospective study, only the paternal parent served in the military. Among nondeployed veterans (two cases of a total 41,345 births examined), only the paternal parent served in the military.

Araneta and colleagues (1997) point out that differences in prevalence rates of Goldenhar syndrome among the offspring of Gulf War veterans (14.7, 95% CI=5.4–36.4) are not significantly different from those of nondeployed veterans (4.8,95% CI=0.8–19.5) because of the small sample sizes and wide confidence intervals. Araneta et al. (1997) determined that, with the sample size maintained as a constant, "the risk would have had to be at least 5.75 times higher among the Gulf War veterans' infants in order to be statistically significant."

As a consequence of this finding of no significance, the Goldenhar syndrome issue was not further pursued in the AEGL analysis for chemical warfare agents. Its utility is further compromised by the fact that there is no confirmed report of veteran exposure to chemical warfare agents, and the Gulf War fathers in the Araneta et al. (1997) study all served in different units and were deployed in theater at different times.

No data are available regarding the potential reproductive and developmental toxicity of agent VX in humans.

2.5. Genotoxicity

There is no information available to evaluate the genotoxicity of G agents or agent VX in humans.

2.6. Carcinogenicity

There are no human data to suggest that G agents or agent VX are carcinogenic.

2.7. Summary

G-series Agents

Available information on the acute inhalation toxicity of agent GB (sarin) to humans is summarized in Table 1–10. Minimal effects observed at low concentrations include miosis, tightness of the chest, rhinorrhea, and dyspnea. The threshold for minimal effects appears to fall in the range of 0.05 to 0.5 mg/m^3 for 10–30 min exposures. The results from different studies are not consistent in identifying the threshold, and that may be due to differences in individual sensitivities, breathing rates of the test subjects, experimental protocols, or analytical methods.

A number of investigators consider *both* miosis and rhinorrhea to be early signs of exposure to cholinesterase inhibitors (Sidell 1997; Mioduszewski et al. 2002b; H. van Helden, Pulmonary and CNS Pharmacology Lab, TNO, the Netherlands, personal communication; S.Tattersall, Biomedical Sciences Division, Porton Down, United Kingdom, personal

TABLE 1–10 Human Experimental Data For GB Vapor (Single Exposures)

Study	GB Concentration (mg/m^3)	Duration	Ct ($mg \cdot min/m^3$)	Signs and Symptoms
Harvey 1952	0.05	20 min	1	Headache, eye pain, rhinorrhea, tightness in chest, cramps, nausea, malaise (*N*=14)
Johns 1952	0.05	20 min	1	Mild miosis (mean maximum decrease in pupil diameter 1–2 mm) in some of the test subjects (150 observations)
McKee and Woolcott 1949	0.06	20 min	1.2	No reported effects (*N*=5)
McKee and Woolcott 1949	0.06	40 min	2.0	"Threshold" for miosis; no other signs or symptoms (*N*=4)
Fairley and Mumford 1948	0.3	0.5 min	0.15	Rhinorrhea in 16 and tightness in chest in 7 (*N*=16)
Baker and Sedgwick 1996	0.5	30 min	1.5	Miosis, dyspnea, photophobia, 40% inhibition of RBC-ChE, changes in SFEMG (*N*=8)
McKee and Woolcott 1949	0.6	1 min	0.6	Miosis and slight tightness in chest (*N*=4)
Rubin et al. 1957	2	2 min	4	Miosis; no other signs of ChE inhibition (*N*=3)
Callaway and Dirnhuber 1971		10 min to 5 h	3.13[a]	50% pupil area decrement

Study	GB Concentration (mg/m^3)	Duration	Ct (mg·min/m^3)	Signs and Symptoms
Callaway and Dirnhuber 1971		10 min to 5 h	13.85[b]	90% pupil area decrement
Oberst et al. 1968	4.19	2 min	8.38	47% inhibition of RBC-ChE; no other effects; eyes not exposed (breathing rate 5.6–8.4 L/min)
Oberst et al. 1968	20.7	2 min	41.4	49% inhibition of RBC-ChE; no other effects; eyes not exposed (breathing rate 47–65 L/min)

Note: Entries are from primary sources and known experimental data
[a]95% confidence limits 2.15–4.57 mg·min/m^3.
[b]95% confidence limits 6.00–32.02 mg·min/m^3.

communication). The presence of rhinorrhea can be indicative of inhalation exposure and/or development of systemic effects, while miosis alone in the absence of other signs or symptoms is a local effect to the pupillary muscles of the eye. As a consequence, the presence of miosis is considered an appropriately sensitive indicator of direct vapor exposure and has the additional advantage of being readily recognized and quantifiable.

There is no evidence that exposure to any of the G agents results in developmental or reproductive toxicity, nor are there any data available to evaluate potential genotoxicity in humans. The G agents have not been identified as human carcinogens.

Agent VX

Experimental data on the effects of acute VX exposures to humans are summarized in Tables 1–11 and 1–12; very few studies have been conducted using exposures to VX vapor, and available data are not of sufficient quality to be used directly in the development of AEGL estimates.

A comparison of the results of the intravenous studies, in terms of the estimated absorbed dose, allows for an evaluation of the dose-response relationship (Table 1–11).

Studies indicate that an intravenous dose of about 1 μg/kg can result in 50% ChE depression and some symptoms of toxicity (headaches); an intravenous dose of about 0.1 μg/kg is unlikely to affect RBC-ChE, but may cause mild effects (headache, chest tightness, dyspnea); and an intravenous dose of 0.01 μg/kg may be below the effects threshold. The estimated equivalent air concentrations for these dose levels, using standard default values for body weight (70 kg) and breathing rate (0.0138 m^3/min), are also listed in Table 1–11. They are highly derivative values and are only presented for comparative purposes.

Experimental data from the Bramwell et al. (1963) study are summarized in Table 1–12. Although the Bramwell et al. (1963) data are considered suspect, they provide a means of comparison with the equivalent concentrations estimated from the intravenous data. Both sets of data suggest that a 10-min exposure to VX at 0.5 mg/m^3 or higher may produce substantial depression of RBC-ChE activity and some clinical signs of toxicity.

Available data summarized suggest that a 10-min exposure to VX at 0.5 mg/m^3 or higher may produce depression of RBC-AChE activity and some clinical signs of toxicity.

TABLE 1–11 Human Experimental Data for VX

Dose (µg/kg)	Exposure Route	Estimated Equivalent Concentration (mg/m^3)[a]	End Point	Reference
0.01–0.13 (estimated)	Inhalation (sniff test)	0.05–3.34	No ChE change; headache, chest tightness, dryness of the mouth	Koon et al. 1959
0.04	Intravenous (30 s injection)	0.41 (0.02 for 10 min)[b]	No ChE change; headache, tiredness, irritability	Kimura et al. 1960
0.12	Intravenous (2 doses over 3.5 h)	0.003 (0.06 for 10 min)[b]	No ChE change; headache, light headedness, abdominal cramps, decrease in respiration and pulse rates, increase in airway resistance and minute volume	Kimura et al. 1960
1.0	Intravenous (over 1.75–4 h)	0.021(0.5 for 10 min)[b]	50–60% depression in ChE activity; headaches in 1/6 individuals	Kimura et al. 1960
1.0	Intravenous (1 dose)	10 (0.51 for 10 min)[b]	50% inhibition of RBC-ChE	Sidell and Groff 1974
1.5	Intravenous (1 dose)	15 (0.76 for 10 min)[b]	75% depression in ChE: dizziness (11/18), nausea (4/18), vomiting (6/18)	Sidell and Groff 1974
1.43	Oral (1 dose/d for 7 d)	NA	60% inhibition of RBC-ChE; no signs or symptoms of toxicity	Sim et al. 1964
2.3	Oral	NA	50% inhibition of RBC-ChE	Sidell and Groff 1974

2–4.5	Oral	NA	Gastrointestinal symptoms in 5/32	Sidell and Groff 1974

Entries are from primary sources and known experimental data

[a]Equivalent concentration estimated from intravenous dose, using as default values 70 kg body weight, and breathing rate of 0.0138 m^3/min (13.8 L/min), and the maximum infusion time listed for the intravenous dose; for single intravenous doses, an estimated time of 30 s was used.

[b]Values in parentheses are for inhalation exposures, standardized to 10 min, using linear extrapolation. For a breathing rate of 0.055 m^3/min (55 L/min) corresponding to heavy activity, the estimated 10-min equivalent concentrations would be approximately one-fourth of the values listed.

TABLE 1–12 Human Experimental Data For VX Vapor from Bramwell et al. (1963)a

VX Concentration (mg/m^3)	Duration	Estimated Ct ($mg \cdot min/m^3$)	Signs and Symptoms
0.2–0.57	3 min	0.6–1.7	10–22% ChE inhibition; slight tightness in throat and upper respiratory tract, some miosis (*N*=7); eyes closed
0.31	3 min	0.9	Sudden miosis in one individual with eyes open
1.6–2.4	1.5 min	2.4–3.6	18–41% ChE inhibition: slight tightness in throat and upper respiratory tract; some miosis (*N*=8); eyes closed
0.8–1.06	6–7 min	4.8–6.4	44–70% ChE inhibition; slight tightness in throat and upper respiratory tract; some miosis (*N*=4); eyes closed

[a]The majority of a Surgeon General's review panel convened by the CDC in Atlanta in August 2000 considered the Bramwell data to be "suspect" and recommended that they not be used in deriving exposure estimates (67 Fed. Reg. 894 [2002]; DHHS 2002). They are presented here for completeness.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

Acute inhalation lethality data for agents GB, GA, GD, GF, and VX for several laboratory species are summarized in Tables 1–13 through 1–17. Additional lethality information is presented in the following subsections.

3.1.1. Nonhuman Primates

Agent GB

The National Defense Research Committee (NDRC) (1946) reported an LCt_{50} value of 150 mg·min/m^3 for 10-min exposure in five monkeys. Cresthull et al. (1959) reported that inhalation doses of GB at 19.2–48.4 μg/kg (0.55-min exposures) resulted in 33–100% mortality in monkeys.

Johnson et al. (1988) conducted a series of lethality studies in which nonhuman primates (baboons) were exposed to GB by the inhalation pathway. Baboons were considered to be a more appropriate test species than rodents or dogs because of the similarities between baboon and human lungs in both biochemical and functional characteristics. Young male baboons were exposed to GB vapor at 1.25–1.3 LD_{50} (N=6) or GD vapor at 2 LD_{50} (N=5). As a result of these tests, Johnson et al. (1988) (see also Anzueto et al. [1990]) reported that inhalation exposures were in close agreement with the reported intravenous LD_{50} values (approximately 13 μg/kg). Woodard et al. (1994) reported an intravenous LD_{50} of 14.7 μg/kg for GB in rhesus monkeys.

Vapor inhalation studies were conducted by Oberst (1961) on monkeys (species not identified) fitted with masks through which GB concentrations were administered. The eyes were protected by eyepieces and were not exposed. The resulting 2-min LCt_{50} value of 42 mg·min/m^3 is reported in Table 1–9.

Agent GA

DA (1974) (secondary source) reported LCt_{50} values of 187 and 135 mg·min/m^3 for monkeys.

TABLE 1–13 Acute Inhalation Lethality Values for Agent GB in Animals (Toxicity Value, LCt50)

Species	Duration (min)	Ct (mg·min/m^3)	Reference
Monkey	10	74	DA 1974
Monkey	2	42	DA 1974
Monkey	0.167	27	DA 1974
Monkey	10	150	NDRC 1946[a]
Monkey	2	42	Oberst 1961[a]
Dog	10	60	DA 1974
Dog	2	56	Oberst 1961[a]
Rabbit	10	120	DA 1974
Guinea pig	1	140	Oberst 1961[a]
Guinea pig	10	180	DA 1974
Rat	5	164 (f)	Mioduszewski et al. 2000, 2001[a]
Rat	10	181 (f)	Mioduszewski et al. 2000, 2001[a]
Rat	30	255 (f)	Mioduszewski et al. 2000, 2001[a]
Rat	60	383 (f)	Mioduszewski et al. 2000, 2001[a]
Rat	90	401 (f)	Mioduszewski et al. 2000, 2001[a]
Rat	240	727 (f)	Mioduszewski et al. 2000, 2001[a]
Rat	360	947 (f)	Mioduszewski et al. 2000, 2001[a]
Rat	5	230 (m)	Mioduszewski et al. 2000, 2001[a]
Rat	10	226 (m)	Mioduszewski et al. 2000, 2001[a]
Rat	30	265 (m)	Mioduszewski et al. 2000, 2001[a]
Rat	60	453 (m)	Mioduszewski et al. 2000, 2001[a]

Species	Duration (min)	Ct (mg·min/m^3)	Reference
Rat	90	433 (m)	Mioduszewski et al. 2000, 2001[a]
Rat	240	982 (m)	Mioduszewski et al. 2000, 2001[a]
Rat	360	1040 (m)	Mioduszewski et al. 2000, 2001[a]
Rat	10	220	DA 1974
Rat	10	300	Cohen et al. 1954[a]
Rat	1	220 (m), 118 (f)	Callaway and Blackburn 1954[a]
Rat	5.0–6.7	191 (f)	Schoene et al. 1985[a]
Mouse	30	501	Bide et al. 1999
Mouse	30	600	Husain et al. 1993[a]
Mouse (active)	10	240	DA 1974
Mouse (resting) 10	310	DA	1974

[a]Italic entries are from primary sources and known experimental data.
Abbreviations: f, female; m, male.

Agent GD

In tests conducted on baboons, Johnson et al. (1988) found that the effects of inhalation exposures were in close agreement with the reported intravenous LD_{50} values of 6.6 μg/kg. Adams et al. (1975) reported a 15-d intramuscular LD_{50} of 6.57 μg/kg.

3.1.2. Dogs

Agent GB

NDRC reported LCt_{50} values of 100–150 mg·min/m^3 for 10-min exposures. Cresthull et al. (1959) reported that inhalation doses of GB at 25.1

and 26.0 μg/kg (0.6- and 2.23-min exposures) were not lethal to dogs, but inhalation doses of GB at 32.5 μg/kg or higher caused 40% or more mortality. Bide et al. (1999) and Yee et al. (1999) developed a three dimensional probit model to calculate lethality values (LC_{05}, LC_{50} LC_{95}) from historic laboratory data and to estimate equivalent human values. Using the species-specific constants for inhalation rates, body mass, et cetera, provided by these authors, the 30-min LC_{50} value for dogs was calculated to be 4.3 mg/m^3.

TABLE 1–14 Acute Inhalation Lethality Values for Agent GA in Animals (Toxicity Value, LCt50)

Species	Duration (min)	Ct (mg·min/m^3)	Reference
Monkey	10	187	DA 1974[a]
Monkey	10	135	DA 1974[a]
Dog	2	320	DA 1974[a]
Rabbit	10	960	DA 1974[a]
Rat	10	450	DA 1974[a]

[a]DA (1974) is a secondary source.

Inhalation studies were conducted by Oberst (1961) on dogs (breed not identified) fitted with masks through which GB vapor flowed. The eyes were protected by eyepieces and were not exposed. The resulting 2-min LCt_{50} value of 56 mg·min/m^3 is reported in Table 1–9.

Agent GA

DA (1974) (secondary source) reported an LCt_{50} value of 320 mg·min/m^3 for dogs.

Agent VX

Bide and Risk (2000) cite several earlier studies in which the LCt_{50} values for VX aerosols were reported to be 15 mg·min/m^3 (whole body) (Krackow 1956) and 15.1 mg·min/m^3 (Punte and Atkinson 1960).

TABLE 1–15 Acute Inhalation Lethality Values for Agent GD in Animals (Toxicity Value, LCt50)

Species	Duration (min)	Ct (mg·min/m^3)	Reference
Rabbit	10	160	DA 1974
Rat	1	196 (m) 135 (f)	Callaway and Blackburn 1954[a]
Rat	5.3–8.5	211 (f)	Schoene et al. 1985[a]
Rat	10	279	DA 1974
Rat	10	230	DA 1974
Rat	<30 (threshold at 16)	400 (threshold at 335)	Aas et al. 1985[a]
Guinea pig	8	480	Langenberg et al. 1998[a]

[a]Entries are from primary sources and known experimental data.
Abbreviations: f, female; m, male.

3.1.3. Rats

Agent GB

NDRC (1946) reported LCt_{50} values of 150–300 mg·min/m^3 for 10-min exposures. Bide et al. (1999) and Yee et al. (1999) developed a three dimensional probit model to calculate lethality values (LC_{05}, LC_{50}, LC_{95}) from historic laboratory data and to estimate equivalent human values. Using the species-specific constants provided, the 30-min LC_{50} value for rats was calculated to be 8.2 mg/m^3.

In studies conducted by Mioduszewski et al. (2000, 2001, 2002a), the acute lethal toxicity of GB to male and female Sprague-Dawley rats was evaluated for time periods of 10, 30, 60, 90, 240 and 360 min in a whole-body dynamic chamber. The final report of this study (Mioduszewski et al. 2001, 2002a) is further documentation of the findings presented below. Ten males and 10 females were used for each concentration-time (Ct) combination, and 50 males and 50 females were used for each time point. GB concentrations ranged from about 2 mg/m^3 to 56 mg/m^3. Agent concentrations were confirmed in the exposure chamber by three procedures ("Edgewood" bubblers, solid sorbent tubes, and a phosphorous monitor) to allow

point and continuous determinations (Mioduszewski et al. 2000, 2001, 2002a). Lethality was assessed at 24 h and at 14 d. Female rats were reported to be more sensitive to GB vapor toxicity than males over the range of exposure concentrations and durations studied (Mioduszewski et al. 2000, 2001, 2002a). Gender differences were reported to be significant at $p<0.01$. Probit analysis (MINITAB, version 13) presented in Mioduszewski et al. (2000) gave the following 14-d LC_{50} values for female rats exposed to GB vapor: 18.1 mg/m^3 for 10 min; 8.51 mg/m^3 for 30 min; 6.39 mg/m^3 for 60 min; 3.03 mg/m^3 for 4 h; and 2.63 mg/m^3 for 6 h.

TABLE 1–16 Acute Inhalation Lethality Values for Agent GF in Animals (Toxicity Value, LCt50)a

Species	Duration (min)	Ct (mg·min/m^3)	Reference
Rat	10	368 (m) 253 (f)	Anthony et al. 2002
Rat	60	396 (m) 334 (f)	Anthony et al. 2002
Rat	240	595 (m) 533 (f)	Anthony et al. 2002

[a]24-h postexposure lethality.
Abbreviations: f, female; m, male.

Probit analysis presented in Mioduszewski et al. (2000) gave the following 14-d LC_{50} values for male rats: 22.6 mg/m^3 for 10 min; 8.84 mg/m^3 for 30 min; 7.55 mg/m^3 for 60 min; 4.09 mg/m^3 for 240 min; 2.89 mg/m^3 for 360 min.

Based on a probit analysis of the data (Mioduszewski et al. 2000), the estimated 14-d LC_{01} values for the females are as follows: 11.54 mg/m^3 for 10 min; 5.84 mg/m^3 for 30 min; 4.01 mg/m^3 for 60 min; 2.09 mg/m^3 for 4 h; 1.76 mg/m^3 for 6 h.

This is the critical study and data set (females) for determination of AEGL-3 values.

GB vapor exposure significantly inhibited rat blood cholinesterase activity in the Mioduszewski et al. (2000, 2001, 2002a) study. However, no correlation between severity of clinical signs and cholinesterase inhibition was reported.

Cohen et al. (1954) reported that exposure of rats to GB at 30 mg/m^3 for 10 min (Ct=300 mg·min/m^3) resulted in mortality rates of close to 50%

and a decrease in brain cholinesterase activity levels to less than 5% of normal.

TABLE 1–17 Lethality in Laboratory Species Following Inhalation Exposure to Agent VX Vapor (End Point, LCt50)

Species	Duration (min)	Ct (mg·min/m^3)	Reference
Mouse (head only)	10 min	13.6	Koon et al. 1960, as cited in NRC 1997
Mouse (whole body)	10 min	4.0	Koon et al. 1960, as cited in NRC 1997
Mouse (nose only)	–	71	Carroll et al. 1957
Mouse (whole body)	–	16.1	Carroll et al. 1957
Goat	10 min	9.2	Koon et al. 1960, as cited in NRC 1997
Mouse	6 h/d, 5 d/wk, 2 wk	0.9	Crook et al. 1983 (noncredible data)
Rat	6 h/d, 5 d/wk, 2 wk	24.9	Crook et al. 1983 (noncredible data)
Guinea pig	6 h/d, 5 d/wk, 2 wk	238.6	Crook et al. 1983 (noncredible data)
Rabbit	6 h/d, 5 d/wk, 2 wk	No deaths	Crook et al. 1983 (noncredible data)

Schoene et al. (1985) reported an LCt_{50} of 191 mg·min/m^3 (95% CI= 178–204 mg·min/m^3) for exposure times of 5.0–6.7 min for female Wistar rats. The corresponding 5-min LC_{50} is 38 mg/m^3. Callaway and Blackburn (1954) reported LCt_{50} values of 220 mg·min/m^3 for male albino rats and 118 mg·min/m^3 for female albino rats (1-min exposures).

Agent GA

DA (1974) (secondary source) reported an LCt_{50} value of 450 mg·min/m^3 for the rat.

Agent GD

In a study designed to secondarily examine agent GD toxicity, Aas et al. (1985) reported that the LCt_{50} for GD in rats (six animals tested at each of three exposure levels for periods of time <30 min) was 400 mg·min/m^3. Aas et al. (1985) graphically present their data as an LCt-versus-mortality curve. The lethality threshold estimated from the curve is about 335 mg·min/m^3. Because the reported GD concentration was fixed at 21 mg/m^3 for the duration of the study, the exposure time corresponding to the lethal threshold is 16 min (see Table 1–11).

The principal objective of the Aas et al. (1985) study was to test an experimental dynamic flow system that would allow study of highly toxic vapors. In consequence, it was necessary to continually generate small amounts of the toxic material in question. Agent GD (soman) was the compound selected to best test the system. Secondary objectives of the study were to determine the (short-term) inhalation toxicity of agent GD (soman) and to study inhibition of acetylcholinesterase, cholinesterase, and carboxylesterase activity in the respiratory tract (relative to other tissues).

Schoene et al. (1985) reported an LCt_{50} of 211 mg·min/m^3 (95% CI= 195–229 mg·min/m^3) for exposure times of 5.3–8.5 min for female Wistar rats. Callaway and Blackburn (1954) reported LCt_{50} values of 196 mg·min/m^3 for male albino rats and 135 mg·min/m^3 for female albino rats (1-min exposures) (Table 1–11).

Agent GF

Callaway and Blackburn (1954) reported LCt_{50} values of 181 mg·min/m^3 for male albino rats and 110 mg·min/m^3 for female albino rats (1 -min exposures). Kassa and Cabal (1999) reported an intramuscular LD50 of 80 μg/kg for rats.

A recent study of GF vapor inhalation toxicity in male and female SD rats reported 24-h postexposure LC_{50} and LCt_{50} values for three exposure periods (10, 60, and 240 min) (Anthony et al. 2002). Young adult rats were exposed whole-body in a dynamic 750-L chamber under protocols similar to those previously published by Mioduszewski et al. (2001, 2002a) but with additional accommodation for the lesser volatility of agent GF. For female rats, Anthony et al. (2002) report 24-h LC_{50} values as follows: 10 min, 25.3 mg/m^3; 60 min, 5.56 mg/m^3; 240 min, 2.22 mg/m^3. For male rats,

24-h LC_{50} values are as follows: 10 min, 36.8 mg/m^3; 60 min, 6.60 mg/m^3; 240 min, 2.48 mg/m^3. These results are summarized as LCt_{50} values in Table 1–16.

Agent VX

Crook et al. (1983) reported an LCt_{50} of 24.9 mg·min/m^3 for animals exposed 6 h/d, 5 d/wk, for 2 wk; however, the results of the study are not considered credible (see Section 3.2).

Bide and Risk (2000) exposed outbred male CD1(SD)BR rats to NaCl aerosols containing entrained VX. The animals (five per test group) were tested with a nose-only inhalation system and for an exposure time of 12 min. Test concentrations were not reported. The observed LCt_{50} was 67 mg·min/m^3.

In studies conducted by Maxwell (1992) on Sprague-Dawley rats, the subcutaneous LD_{50} for VX was reported to be 0.027 μmol/kg. DA (1974) (secondary source) reports intragastric LD_{50} values of 0.1 mg/kg and 0.077–0.1280 mg/kg for rats.

3.1.4. Mice

Agent GB

NDRC (1946) reported LCt_{50} values of 150–300 mg·min/m^3 for 10-min exposures, 360 mg·min/m^3 for a 20-min exposure, and 420 mg·min/m^3 for a 30-min exposure (14 mg/m^3 for 30 min). Clement (1992) reported a subcutaneous LD_{50} value of 170 μg/kg for male CD-1 mice with body weight ranging from 30–40 g (five animals per test group). A subcutaneous LD_{50} of 0.212 mg/kg was reported for male and female Shanghai mice (18–22 g body weight, eight mice per group, five exposure groups) (Luo and Liang 1997). Bide et al. (1999) and Yee et al. (1999) developed a three dimensional probit model to calculate lethality values (LC_{05}, LC_{50}, LC_{95}) from recently conducted laboratory experiments on mice and historic toxicity data for other laboratory species and to estimate equivalent human values. Using the species-specific constants provided by Bide et al. (1999), the 30-min LC_{50} value for the mouse was calculated to be 16.7 mg/m^3.

The LCt_{50} for agent GB in female Swiss albino mice has been reported

to be 600 mg·min/m^3 (Husain et al. 1993), equivalent to a 30-min exposure at 20 mg/m^3. Lohs (1960) reported a 30-min inhalation lethality value of value of 5 mg/m^3.

Agent GD

Lohs (1960) reported a 30-min inhalation lethality value of 1 mg/m^3.

Agent GF

Inhalation lethality data for agent GF were not found in the available literature. Luo and Liang (1997) reported an LD_{50} of 0.346 mg/kg in mice injected with the agent subcutaneously. Clement (1992) reported a subcutaneous LD_{50} value of 243 μg/kg for male CD-1 mice with body weights ranging from 30–40 g (five animals per test group).

Agent VX

Ten-minute LCt_{50} values of 4.0 mg·min/m^3 (whole body) and 13.6 mg·min/m^3 (head only) have been reported for mice exposed to VX vapors (Table 1–6) (Koon et al. 1960, as cited in NRC 1997). LCt_{50} values of 71 mg·min/m^3 for female mice for nose-only exposures and 16.1 mg·min/m^3 for whole-body exposures were reported by Carroll et al. (1957) for female mice; however, in this study it was reported that the concentration of the agent in the exposure chamber was not measured directly but was estimated from the mortality level, which was correlated with the LD_{50} for an intravenous injection (estimated to be 17 μg/kg).

Crook et al. (1983) reported an LCt_{50} of 0.9 mg·min/m^3 for animals exposed 6 h/d, 5 d/wk, for 2 wk to VX vapors; however, the results of this study are not considered credible.

Koplovitz et al. (1992) exposed Swiss ICR mice intramuscularly to GB and VX. The resulting acute (24-h) LD_{50} are as follows: LD_{50} for GB of 204.81 μg/kg, LD_{50} of VX of 13.07 μg/kg.

Bide and Risk (2000) exposed outbred male CD1(ICR)BR mice to NaCl aerosols containing entrained VX. The animals (five per test group) were tested with a nose-only inhalation system and for a exposure time of

12 min. Test concentrations were not reported. The observed LCt_{50} was 72 mg·min/m^3. Bide and Risk (2000) also cite several earlier studies in which the LCt_{50} values for VX aerosols were reported to be 7 mg·min/m^3 (Krackow 1956) and 6.1 mg·min/m^3 (Punte and Atkinson 1960).

3.1.5. Guinea pigs

Agent GB

NDRC (1946) reported LCt_{50} values of 150–250 mg·min/m^3 for 10-min exposures. Bide et al. (1999) and Yee et al. (1999) developed a three dimensional probit model to calculate lethality values (LC_{05}, LC_{50}, LC_{95}) from historic laboratory data and to estimate equivalent human values. Using the species-specific constants provided, the 30-min LC_{50} value for guinea pigs was calculated to be 7.5 mg/m^3.

Oberst (1961) conducted inhalation studies on guinea pigs administered GB vapor by means of face masks "which filled the face without leakage." The author does not mention eye protection for guinea pigs. The resulting 1-min LCt_{50} of 140 mg·min/m^3 is reported in Table 1–9.

Atchison et al. (2001) reported that subcutaneous injections of 0.6 LD_{50} GB once per day, 5 d/wk, in young male Hartley guinea pigs (600 g) resulted in 50% mortality (two of four) after 2 wk of exposure and 100% mortality after 3 wk. The subcutaneous LD_{50} for guinea pigs was reported to be 42 μg/kg.

Agent GD

Allon et al. (1998) reported an inhalation LD_{50} of 101 μg/kg for guinea pigs, considerably higher than the reported intravenous LD_{50} values of 22 μg/kg (Sterri et al. 1982) and 3.5 μg/kg (Due et al. 1993). For guinea pigs weighing 0.84 kg and breathing 0.4 m^3/d (EPA defaults), a dose of 101 μg/kg would be equivalent to an exposure to GD at 0.009 mg/m^3 for 24 h, 0.22 mg/m^3 for 1 h, or 0.44 mg/m^3 for 30 min. Langenberg et al. (1998) reported an 8-min LCt_{50} of 480 mg·min/m^3 for guinea pigs. This value is equivalent to an LC_{50} of 60 mg/m^3 for 8 min or 16 mg/m^3 for 30 min (assuming linear scaling).

Atchison et al. (2001) reported that subcutaneous injections of 0.6 LD_{50}

GD once per day, 5 d/wk, in young male Hartley guinea pigs (600 g) resulted in 50% mortality (two of four) after 2 wk of exposure and 100% mortality after 9 wk. The subcutaneous LD_{50} for guinea pigs was reported to be 28 μg/kg.

Agent VX

Crook et al. (1983) reported an LCt_{50} of 238 mg·min/m^3 for animals exposed 6 h/d, 5 d/wk, for 2 wk; however, the results of this study are not considered credible.

Koplovitz et al. (1992) exposed Hartley albino guinea pigs (subcutaneous) to GB and VX. The resulting acute (24-h) LD_{50} are as follows: LD_{50} for GB of 41.26 μg/kg, LD_{50} for VX of 6.89 μg/kg.

Bide and Risk (2000) exposed outbred male (HA)BR guinea pigs to NaCl aerosols containing entrained VX. The animals (five per test group) were tested with a nose-only inhalation system and for an exposure time of 12 min. Test concentrations were not reported. The observed LCt_{50} was 30 mg·min/m^3. Bide and Risk (2000) also cite several earlier studies in which the LCt_{50} values for VX aerosols were reported to be 30 mg·min/m^3 (whole body) (Krackow 1956) and 29.5 mg·min/m^3 (Punte and Atkinson 1960).

Atchison et al. (2001) reported that subcutaneous injections of 0.6 LD_{50} VX once per day, 5 d/wk, in young male Hartley guinea pigs (600 g) resulted in 33% mortality (two of six) after 10 wk of exposure and 83% mortality (five of six) after 9 wk. One animal survived the full 13 wk of treatment. The subcutaneous LD_{50} for guinea pigs was reported to be 9 μg/kg.

3.1.6. Rabbits

Agent GB

NDRC (1946) reported LCt_{50} values of 120–250 mg·min/m^3 for 10-min exposures. Bide et al. (1999) and Yee et al. (1999) developed a three dimensional probit model to calculate lethality values (LC_{05}, LC_{50}, LC_{95}) from historic laboratory data and to estimate equivalent human values. Using the species-specific constants provided, the 30-min LC_{50} value for rabbits was calculated to be 5.6 mg/m^3.

Agent GA

DA (1974) (secondary source) reported an LCt_{50} of 960 mg·min/m^3 for a 10-min exposure.

Agent VX

Bide and Risk (2000) cite an earlier study in which the LCt_{50} values for a VX aerosol was reported to be 22.1 mg·min/m^3 (Punte and Atkinson 1960).

3.1.7. Cats

NDRC (1946) reported an LCt_{50} value of 100 mg·min/m^3 for a 10-min exposure. Bide et al. (1999) and Yee et al. (1999) developed a three dimensional probit model to calculate lethality values (LC_{05}, LC_{50}, LC_{95}) from historic laboratory data and to estimate equivalent human values. Using the species-specific constants provided by Bide et al. (1999), the 30-min LC_{50} value for cats was calculated to be 3.9 mg/m^3.

3.1.8. Goats

Agent VX

A single 10-min LCt_{50} of 9.2 mg·min/m^3 has been reported for goats (Table 1–6) (Koon et al. 1960, as cited in NRC 1997).

3.1.9. Hamsters

Agent VX

Bide and Risk (2000) cite several earlier studies in which the LCt_{50} values for VX aerosols were reported to be 17 mg·min/m^3 (whole body) (Krackow 1956) and 14.7 mg·min/m^3 (Punte and Atkinson 1960).

3.1.10. Summary of Acute Lethality Data in Animals

Acute inhalation lethality data for agents GB, GA, GD, GF, and VX for several laboratory species are summarized in Tables 1–13 through 1–17.

In addition to the data presented in Sections 3.1 through 3.9, Tables 1– 13 through 1–17 contain data obtained from several historical reference handbooks (NDRC 1946; DA 1974). The DA (1974) reference source also contains LCt_{50} values for exposure times less than 10 min (i.e., from 2 s to 2 min). Christensen et al. (1958) used data sets for agent GB to develop LCt_{50}-exposure time curves for each species. The original sources of the lethality data are cited by Christensen et al. (1958). A similar data set (exposures from 2 s to 12 min) was also used by Yee (1996) (see also Yee et al. [1999] and Bide et al. [1999]) who evaluated the relationship between lethal concentrations and exposure times.

3.2. Nonlethal Toxicity

3.2.1. Nonhuman Primates

Agent GB

Increases in high frequency beta activity were observed by Burchfiel and Duffy (1982) in the EEGs of rhesus monkeys who had been injected with agent GB (sarin) 1 y earlier (with either a single 5 μg/kg intravenous dose or with a series of intramuscular injections of 1 μg/kg, given once per week for 10 wk). Control animals did not show any changes in EEG. Neurobehavioral tests were not conducted on the exposed animals. In a similar series of tests in which marmosets were injected intramuscularly with 3 μg/kg, a slight but nonsignificant increase in beta activity was observed 15 mo after the exposure (Pearce et al. 1999). Behavioral tests indicated no deleterious effects on cognitive performance. RBC-ChE activity was reduced by 51.3% in the dosed animals.

Christensen et al. (1958) cite several earlier studies (Cresthull et al. 1957; Callaway and Crichton 1954) in which the incapacitation Ct_{50} for GB for monkeys was reported to be 67–75% of the LCt_{50} value. The ICt_{50} value is estimated to be 47 mg·min/m^3 for a 2-min exposure (derived from the graphic presentation of the data given by Christensen et al. [1958]). Incapacitation was defined as convulsions, collapse, or death. Anzueto et al. (1990) reported that inhalation of 30 μg/kg (2 times the LD_{50}) by four

baboons resulted in cardiac arrhythmias, apnea, and a significant decrease in mean blood pressure. Single intramuscular injections of 6 μg/kg to marmosets resulted in adverse behavioral effects when the animals were tested for hand-eye coordination, but no adverse effects were seen in a visual discrimination test (Wolthuis 1992). A dose of 3 μg/kg had no adverse effects on behavior, and hand-eye coordination was improved (versus each individual animal's baseline performance prior to exposure) in three of six animals (Wolthuis 1992).

Ashwick and deCandole (1961) reported that subcutaneous GB doses of greater than 0.04 mg/kg would result in convulsions in monkeys.

van Helden et al. (2001, 2002) exposed nearly equal numbers of male and female marmosets (*Callithrix jacchus,* Harlan, United Kingdom) (whole-body) to GB vapor concentrations at 0.05 to 150 μg/m^3 for 5 h. The lowest cumulative exposure at which the internal dose became measurable (based on fluoride-regenerated GB from blood BuChE) was 0.04±0.01 mg·min/m^3 (N=5). The LOAELs for miosis, EEG effects, and visual evoked response (VER) were determined by testing one animal at each of the following concentrations: 7.5, 15, 25, 50, and 150 μg/m^3. Controls (N =5) were exposed to air for 5 h. For miosis, the LOAEL (10% decrease in pupil size compared with controls; estimated at approximately 20% decrement in pupil area; p<0.05) was reported to be 2.5±0.8 mg·min/m^3. The LOAEL (p<0.05) for changes in EEG parameters was 0.2 mg·min/m^3 (indicative value), and the LOAEL for VER was 25 mg·min/m^3 (indicative value). The blood AChE activity for marmosets was significantly (p< 0.05) inhibited at all GB vapor exposure concentrations and exhibited dose dependence. Although van Helden et al. (2001, 2002) reported that the EEG signal appeared to be more sensitive to GB than the eye, they noted that the EEG effects are more complex and concluded that "the miotic response, showing a clear dose-relationship, might therefore be considered at this moment as the most reliable biomarker of exposure to low levels of GB."

Agent GD

Anzueto et al. (1990) reported that inhalation of GD at 13.14 μg/kg (2 times the LD_{50}) by five baboons resulted in cardiac arrhythmias, apnea, and a significant decrease in mean blood pressure. Lipp and Dola (1980) reported that intramuscular injections of 30–75 μg/kg would result in seizure activity and convulsions in female rhesus monkey.

3.2.2. Dogs

Agent GB

Harris et al. (1953) exposed four mongrel dogs in a chamber to an average Ct of 10.5 mg·min/m^3 for an exposure duration of 20 min/d (equivalent to an average concentration of 0.53 mg/m^3), 5 d/wk for 2 mo. The only reported clinical sign was miosis, which appeared with each exposure but disappeared prior to the next exposure. However, when each daily exposure was increased to 15 mg·min/m^3, toxic signs (body tremors, dyspnea, loss of muscle control, convulsions) occurred within 7–10 d and several dogs died. When the Ct was again reduced to 10 mg·min/m^3, all signs but miosis disappeared and RBC-AChE stabilized at a level between zero and 20% of normal in the surviving dogs. Sixty-one percent of the total blood ChE activity in dogs is found in the RBC (Osweiler et al. 1985).

Fogleman et al. (1954) exposed three beagle dogs (average body weight 11.4 kg) to agent GB (sarin) vapors (face-only) for three successive exposure periods (4, 6, and 6 wk) with intervening time periods to allow for complete recovery of RBC-AChE (recovery times not reported). During each test period, the animals were exposed for time periods ranging from 8–24 min/d for 5 d/wk. In the first exposure period (series I), a concentration of 0.24–0.26 mg/m^3 for 8, 16, or 24 min/d for a total of 17–20 exposures produced only mild salivation and rhinorrhea. These effects were thought to be due to the type of mask used on the animals (the Snell dog mask). RBC-AChE activity was not recorded. In series II, in which the Saunders-Fogleman dog mask was used, 39 exposures over 6 wk at a concentration of 0.73–0.75 μg/L (0.73–0.75 mg/m^3) for 8, 16, or 24 min/d produced dyspnea when the daily total amount of agent GB (sarin) retained exceeded 2 μg/kg. One of the three dogs in series II exhibited gluteal muscle fasciculations when the total retained dose reached 64.5 μg/kg (after about 23 exposures). In series III (exposures at 2.38–2.43 mg/m^3 for 8, 12, or 16 min/d and a total of 30 exposures over 6 wk), dyspnea and fasciculations in the region of the gluteal muscle occurred when the daily retained dose exceeded 2 μg/kg. RBC-AChE activity of all three dogs dropped to approximately 65% of normal (percent of preexposure value) after the first exposure. After the fourth exposure, RBC-AChE activity in the dog exposed for 16 min/d dropped to zero while the RBC-AChE activity in the other two dogs was about 35% of normal (range of 32–38% for the 12-min and 8-min exposure animals, respectively). Analysis of expired air allowed

for the estimation of the total amount of agent GB (sarin) retained in the body; average measured retention rates ranged from 78.9% to 84.3% in the series III tests.

Jacobson et al. (1959) exposed male beagle dogs (three per group) at 0.04 mg/m^3, 4 h/d, 5 or 7 d/wk for 6 mo or at 0.50 mg/m^3, 20 min/d, 5 or 7 d/wk for 6 mo. The lowest test concentration of 0.04 mg/m^3 for 4 h/d resulted in decreased RBC-AChE activity (to less than 30% of the baseline values), miosis, dyspnea, increased salivation, and rhinorrhea. The effects were more severe in animals exposed 7 d/wk rather than 5 d/wk and in animals exposed to the higher concentration for 20 min/d rather than the lower concentration for 4 h/d. Miosis persisted throughout the entire 6-mo test period. Jacobson et al. (1959) autopsied two dogs in each exposure group at the end of the 6-mo period and found "some thickening of the musculature of the bronchioles and alveolar duct…dilation of the mucous glands in the bronchial trees and some flattening of the epithelium…(and) …some emphysematous areas and interstitial pneumonitis." Histopathology of other organs was not reported. Brain ChE activity (measured in one dog per exposure group and in one control at autopsy, by the manometric method of Ammon [1933] as modified by Cohen et al. [1954]) was not significantly affected by the GB exposure except possibly in the dog exposed at 0.5 mg/m^3, 20 min/d, 7 d/wk. In the latter case, brain ChE activity was 45% of the control value. Weekly electrocardiograms (EKGs) did not show any changes in the exposed animals except those that might be associated with hypoxia. Hematological counts showed no significant changes; clinical chemistry was not reported.

Weimer et al. (1979) exposed purebred beagle dogs at 0, 0.0001, or 0.001 mg/m^3 for 6 h/d, 5 d/wk, for up to 52 wk. Four male and eight female beagles were exposed to each test concentration; however, only two females per exposure group were exposed for the full 52-wk period. In the exposed animals, statistically significant changes in RBC-AChE activity occurred occasionally (blood samples drawn after 1 and 2 wk of exposure, and thereafter on a monthly basis). However, these changes did not follow a clear dose-response or duration-response pattern. Two of 12 dogs exposed at 0.0001 mg/m^3 and three of 12 exposed at 0.001 mg/m^3 exhibited abnormal EKGs at the time of sacrifice (one each at 4, 12, and 52 wk and two at 24 wk); elevated P waves were suggestive of right atrial hypertrophy. However, there was no evidence of enlargement or physical abnormalities of the heart. Weimer et al. (1979) noted that the anomalies could have been preexisting conditions. Baseline EKGs, which were available only for

four dogs exposed for 2 mo and for four dogs exposed for 36 wk (and surgically modified to allow for periodic physiological measurements), did not reveal any evidence of EKG abnormalities. The absence of baseline data for the other test animals precludes identifying the reported EKG changes as being caused by the GB exposure, and statistical analysis of the data is not possible because of the small number of test animals (only two animals per exposure group were tested for each exposure duration).

3.2.3. Rats

Agent GB

Kassa et al. (2001) exposed male albino Wistar rats for 60 min in an inhalation chamber once or repeatedly to GB concentrations at 0.8, 1.25, or 2.5 mg/m^3. The lowest concentration (level 1) was reported to be asymptomatic based on clinical and laboratory measurements. The second concentration (level 2) was reported to be asymptomatic based on clinical signs but produced a significant inhibition of RBC-AChE (by 30%). The level 2 concentration was tested using a single exposure or three exposures during 1 wk. The highest test concentration (level 3) was reported to be a nonconvulsive symptomatic exposure. Controls were exposed to pure air only. Three months following the exposure, the control and exposed animals (10 per test group) were evaluated for GB-induced effects using biochemical, hematological, neurophysiological, behavioral, and immunotoxicological methods. None of the exposed animals showed any clinical signs of intoxication 3 mo after exposure; their body weights did not differ significantly from control values, and there were no changes in hematological or biochemical parameters, including blood and brain cholinesterase. Test animals exposed at 0.8 mg/m^3 (level 1) for 60 min showed no alterations in immune function, as measured by in vitro spontaneous or lipopolysaccharides-stimulated proliferation of spleen cells, or by in vitro evaluation of the production of reactive nitrogen intermediates (N-oxides), indicative of bactericidal efficacy of peritoneal macrophages. Level 1 test animals also showed no neurotoxic effects after 3 mo when monitored using a functional observatory battery (FOB) and a test of excitability of the CNS on the basis of the observation of convulsive activity after intraperitoneal administration of pentamethylenetetrazol. The only significant effect (p<0.05) observed in rats exposed once to 1.25 mg/m^3

was an increase in stereotyped behavior. Effects observed in rats exposed three times to 1.25 mg/m^3 included a significant increase ($p<0.05$) in the excitability of the CNS, significant alterations of mobility score ($p<0.01$) and gait disorder ($p<0.001$) characterized by ataxia, and a significant increase in stereotyped behavior ($p<0.001$). Animals exposed once to 2.5 mg/m^3 exhibited significant changes in some immune functions ($p<0.05$), mobility score ($p<0.01$), activity ($p<0.01$), gait score ($p<0.01$), gait disorder ($p<0.001$), and stereotyped behavior ($p<0.01$).

Henderson et al. (2000, 2001, 2002), Conn et al. (2002), and Kalra et al. (2002) exposed male F344 rats at 0.0, 0.2, or 0.4 mg/m^3 (nose-only) for 1 h/d for 1 d, 5 d, or 10 d, with sacrifices at 1 d after exposure and at 1 mo after exposure. Tests were conducted under normal temperatures (25 °C) and under heat stress (32 °C) conditions (core body temperature raised 1 °C). Study parameters included overt symptoms of toxicity, body temperature and activity, body weight, breathing patterns, cytokine levels in brain, Con-A-stimulated mitogenesis in splenic lymphocytes, number of cholinergic receptor sites in brain, and apoptosis in brain cells. It is reported that no overt symptoms of neurotoxicity (tremors) occurred at either exposure level after a single, 1-d exposure. Single exposures were associated with little inhibition of RBC cholinesterase activity (inhibition of "7 and 11% for the low- and high-exposure groups, respectively") (Henderson et al. 2002); after the 10-d exposure, RBC-ChE activity was reduced 60% for the high-exposure group. Inhibition of plasma cholinesterase activity in heat-stressed animals was greater (approximately 20%) following a single exposure than after repeated exposures (no significant activity changes after 10 d; $p\leq0.05$) (Henderson et al. 2002). Cholinesterase changes measured after single exposures were not associated with clinical signs. Repeated exposures induced some signs of suppression of the immune system in terms of reduced ability to maintain body temperature, and a dose-dependent reduction in the response of splenic lymphocytes to mitogens was recorded. In addition, dose-dependent induction of cytokine expression (IL-1β, IL-6, and TNF-α) was observed in the brain. No signs of increased apoptosis were seen in any of the rats exposed for 1, 5, or 10 d. Further, heat stress in combination with sarin exposure led to an increase in the number of M3 receptor sites in olfactory and adjacent areas of the rat brain. Repetitive heat stress alone reduced body weight gain; sarin exposure did not affect body weights (Henderson et al. 2002).

To better characterize the relationship between miosis and GB vapor exposure concentration and duration, Mioduszewski et al. (2002b) exposed

young adult (8–10 wk) male and female Sprague-Dawley rats to GB vapor at a range of concentrations (0.01–0.48 mg/m^3) and three time durations (10, 60, and 240 min). A total of 283 rats (142 female, 141 male) were exposed whole-body "to GB vapor in a 750-L dynamic airflow inhalation chamber" following published protocols (Mioduszewski et al. 2001, 2002a). Including range-finding experiments and controls (N=130), a total of 423 rats were employed in this well-conducted study. Approximately 30 min postexposure, rat pupil diameters were assessed by means of individual examination with a simple microscope fitted with a reticule eyepiece. Blood samples were also collected (24 h preexposure, 60 min postexposure, and 7 d postexposure at sacrifice) from tail vein and heart (postmortem only) for RBC and plasma carboxylesterase (CaE) and cholinesterase activity determinations using a modified Ellman method (Ellman et al. 1961). Animals were also observed for development of clinical signs during 7 d postexposure. The miosis data were used to generate EC_{50} and ECt_{50} values for both genders for each of the three exposure durations (female EC_{50} was 0.068 mg/m^3 for 10 min, 0.020 mg/m^3 for 60 min, and 0.012 for 240 min or 0.68 mg·min/m^3 for 10 min, 1.20 mg·min/m^3 for 60 min, and 2.88 mg·min/m^3 for 240 min) (male EC_{50} was 0.087 mg/m^3 for 10 min, 0.030 mg/m^3 for 60 min, and 0.024 mg/m^3 at 240 min or 0.87 mg·min/m^3 for 10 min, 1.80 mg·min/m^3 for 60 min, and 5.76 mg·min/m^3 at 240 min). The Mioduszewski et al. (2002b) study defined the EC_{50} and ECt_{50} points as the statistical concentration (or cumulative exposure [Ct]) required for postexposure pupil diameters of 50% or less of the pre-exposure pupil diameter in 50% of the exposed population. Gender differences (females more susceptible) were statistically significant at 10 min (p=0.014) and 240 min (p=0.023), but not at 60 min (p=0.054). Whole-body exposure to GB vapor did not result in significant activity inhibition for any blood enzyme monitored—RBC-AChE, plasma BuChE, or CaE for any GB vapor concentrations and duration tested. The authors conclude that "observable clinical signs associated with whole-body GB vapor exposure can be limited to miosis, even in the absence of significant changes in AChE, BuChE, or CaE activity" (Mioduszewski et al. 2002b, p. 21).

This is the critical study and data set (female Sprague-Dawley rats) for determination of AEGL-1 values for agent GB.

In tests conducted by Cohen et al. (1954), hypertonicity and hyperactivity of the musculature, increased response to stimuli, rigidity, and convulsions were seen in some test animals exposed at 50 mg·min/m^3 (1 mg/m^3 for 50 min, daily). Brain cholinesterase activity became depressed only after

erythrocyte cholinesterase activity had dropped to about 30% of the normal levels (after approximately 58 d exposure at a Ct of 75 mg·min/m^3). All rats exposed to an LCt_{50} at 300 mg·min/m^3 for 10 min had brain ChE values below 5% of normal.

Noninhalation studies have demonstrated that single exposures to GB can result in neurobehavioral changes. An intraperitoneal dose of 50 μg/kg resulted in decreases in rearing and grooming behavior and locomotive activity in male Wistar rats (Nieminen et al. 1990). A subcutaneous injection of 61 μg/kg increased spontaneous motor activity in male Sprague-Dawley rats; a dose of 71 μg/kg produced conditioned flavor aversions; 84 and 115 μg/kg caused significant decreases in spontaneous locomotive activity; and doses of 98 and 115 μg/kg resulted in significant decrements in rotorod performance. At exposures ≤84 μg/kg, no significant effects in rotorod performance were observed (Landauer and Romano 1984). Male Sprague-Dawley rats exposed to a single 100 μg/kg intramuscular dose of GB (LD_{50}) showed significant inhibition of cholinesterase in brain and blood plasma and an increase in choline acetyl transferase activity in cortex and brain stem, but not in the mid-brain (Khan et al. 2000a,b). Olson et al. (2000) reported that subcutaneous doses of GB (once per day for 4 d) sufficient to lower whole blood cholinesterase by 20–30% caused no neurobehavioral or neuropathologic effects in rats. That finding is consistent with what Cohen et al. (1954) reported above.

Young et al. (2001) evaluated the correlation of blood cholinesterase levels with sarin-induced toxicity in female, nonpregnant CD rats (Crl:COBS CD [SD BR Rat Outbred]) treated by gavage with type I sarin at 380 μg/kg once per day for 10 d. Based on the results of previous studies, a dose of 380 μg/kg was expected to result in 30% mortality. Baseline blood cholinesterase values were determined before treatment. After the first dose, there was a drop in plasma cholinesterase which remained low throughout the 10-d test period. A statistically significant correlation ($p< 0.0001$) was found between body weight loss and plasma cholinesterase levels during the period of dosing. However, RBC-cholinesterase levels were not different between control and treated animals. Neither plasma nor RBC-AChE baseline cholinesterase activity levels nor the relative or absolute decline in cholinesterase values could be used as predictors of mortality in the treated animals.

Abu-Qare and Abou-Donia (2001) examined the ability of a single intramuscular dose of agent GB (80 μg/kg) alone, or in combination with a single oral dose of pyridostigmine bromide, to induce markers of oxida

tive stress. Urine samples of treated and control adult SD rats were collected at various times post-treatment (16–96 h). No increase in the concentrations of stress markers 3-nitrotyrosine and 8-hydroxy-2'-deoxyguanosine was detected following a single dose of sarin.

Jones et al. (2000) investigated potential subchronic neurotoxic effects of GB concentrations administered in fractions of the LD_{50} dose (intramuscular at 0.01, 0.1, 0.5, and $1 \times LD_{50}$) to male Sprague-Dawley rats, after which the rats were maintained for 90 d. Potential changes in blood-brain barrier (BBB) permeability were monitored in the cortex, brainstem, midbrain, and cerebellum; other parameters monitored included plasma butyrylcholinesterase activity as well as m2-selective muscarinic acetylcholine receptor (m2-mAChR) and nicotinic acetylcholine receptor (nAChR) ligand binding. Plasma butyrylcholinesterase activity recovers rapidly and cannot, therefore, serve as a reliable biomarker for potential long-term toxicity of sarin exposure. Ninety days after single sarin exposure, changes in brain regional binding densities of the two receptors were noted; the clinical significance of those changes was not reported.

In a subchronic inhalation study conducted on Fischer 344 rats, no signs of toxicity were observed in animals exposed to GB at 0.0001 or 0.001 mg/m^3 6 h/d, 5 d/wk (excluding holidays), for up to 24 wk (Weimer et al. 1979). In a continuation of these studies, Sprague-Dawley/Wistar (colony) and Fischer 344 rats were exposed at 0, 0.0001, or 0.001 mg/m^3 6 h/d, 5 d/wk, for up to 52 wk (Weimer et al. 1979). Fifty animals of each gender of each strain were exposed to each test concentration, and blood samples were drawn for RBC-ChE determination at the time of sacrifice. During a 3-wk period, the test animals exhibited heat stress due to loss of chamber temperature control (temperatures exceeded 90 °F) and many of the rats died (16 in the low exposure group and 12 in the high exposure group). Fluctuations in blood chemistries (including RBC-AChE) for the exposed animals were no greater than controls, and although statistically significant changes in RBC-AChE occurred occasionally, the changes did not follow a clear dose-response or duration-response pattern. Atrophy of the seminiferous tubules was observed in Fischer 344 rats exposed to GB; however, Weimer et al. (1979) noted that this inbred strain of rat is susceptible to numerous genetically based defects that may appear under experimental conditions of stress. The tests were repeated using the same experimental protocol for 12 and 24 wk, and none of the exposed rats in the second assay exhibited testicular atrophy. A high incidence of tracheitis oc

curred in both the Fischer rats and in the colony rats exposed to GB. The most severe cases reportedly occurred in the high-exposure group. The incidence of tracheitis in colony rats is summarized in Table 1–18; the results were analyzed statistically using the Fisher Exact Test (statistical analysis was not provided by Weimer et al. [1979]). Although there were statistically significant differences between exposed and control groups after 4, 8, and 12 wk of exposure, the differences were not significant for longer exposure durations. A similar response was seen in Fischer rats (i.e., a few cases of tracheitis early in the study, but none in animals exposed for 52 wk).

Tracheitis was often common in animal colonies during the time of the Weimer et al. (1979) study and is now considered reflective of incomplete infectious-disease control in the colony. This evidence of disease, coupled with the loss of chamber temperature control and subsequent heat-stress deaths of test animals, compromise the results and disqualify Weimer et al. (1979) from use as a critical study for AEGL estimation.

Agent GD

Walday et al. (1991) exposed male Wistar rats to GD at 0.05 or 0.2 mg/m^3 for a single 40-h period. No clinical signs of toxicity were seen during the exposures. Acetylcholinesterase and butyrylcholinesterase were significantly inhibited in all tissues except the brain.

Agent GF

A recent study of lethal GF vapor exposure toxicity in male and female SD rats also reported sublethal clinical signs of tremors, convulsions, salivation, and miosis following whole-body dynamic chamber exposures (Anthony et al. 2002). Blood samples were also drawn for BuChE activity determinations. A range of near-lethal vapor concentrations were employed for three exposure durations (10, 60, and 240 min). Because the experimental protocol was designed for LC_{50} lethality-effects determination, effective concentration determinations (EC_{50}, ECt_{50}) for nonlethal effects were not estimated by Anthony et al. (2002). Miosis was observed in all exposed rats during the first hour postexposure; the effect was reversible

and pupil sizes were normal at 14 d postexposure. Preliminary analysis of BuChE activity indicates statistically significant depression "immediately after exposure" and statistically significant elevations at 14 d postexposure to near-lethal vapor concentrations; at neither time period is the BuChE delta correlated with cumulative exposure (Ct) (Anthony et al. 2002).

TABLE 1–18 Incidence of Tracheitis in Colony Rats Exposed to Agent GBa

	Exposure Group		
Exposure Period	Control	0.0001 mg/m^3	0.001 mg/m^3
4 wk	0/10	5/10[b]	0/10
8 wk	0/10	4/10[b]	9/10[c]
12 wk	0/10	5/8[c]	5/7[c]
16 wk	0/9	0/10	1/10
20 wk	0/10	0/5	2/6
24 wk	1/10	1/5	0/6
36 wk	0/9	2/5	2/7
52 wk	2/10	1/10	6/10
6 mo	0/19	7/19[c]	9/28[c]

[a]Statistical analysis using the Fisher Exact Test.
[b]Significantly different from control, $p<0.05$.
[c]Significantly different from control, $p<0.01$; the postexposure population was made up of groups of each rodent strain held for 6-mo observation after the experimental exposure period ended.
Source: Weimer et al. 1979.

Agent VX

Crook et al. (1983) conducted VX vapor exposure studies in male and female Sprague Dawley rats. Crook and his colleagues consider their results to be nonverifiable and suspect for the reasons outlined earlier. These data are thus considered too unreliable for any application to development of AEGL estimates for agent VX.

3.2.4. Mice

Agent GB

Signs suggestive of delayed neuropathy have been observed in female Swiss albino mice (N=6) exposed to GB at 5 mg/m^3 for 20 min daily for 10 d (Husain et al. 1993). Muscular weakness of the limbs and slight ataxia occurred on the day 14 after the start of the exposures (the number of animals showing these effects was not specified). Significant (p<0.001) inhibition of NTE activity in the brain (59.2%), spinal cord (47.4%), and platelets (55.4%) was observed in the test animals (N=6). Histological examination of the spinal cord revealed focal axonal degeneration that was reported to be moderate in two animals and light in four. The same exposure inhibited blood AChE by 27.3% and brain AChE by 19.2% but was not associated with any anti-AChE symptoms. The LCt_{50} for this strain of mice was reported to be 600 mg·min/m^3 (Husain et al. 1993), presumably for a 1-min exposure.

Agent VX

Crook et al. (1983) conducted VX vapor exposure studies in male and female ICR mice. Crook and his colleagues consider their results to be nonverifiable and suspect for the reasons outlined earlier. The data are thus considered too unreliable for any application to development of AEGL estimates for agent VX.

3.2.5. Guinea pigs

Agent GB

Van Helden et al. (2001, 2002) exposed male Dunkin-Hartley albino (HSD-Harlan [Harlan]) guinea pigs (whole-body) to GB vapor concentrations at 0.05 to 150 μg/m^3 for 5 h. The lowest cumulative exposure at which the internal dose became measurable (based on fluoride-regenerated GB from blood BuChE) was 0.010±0.002 mg·min/m^3 (N=12). The LOAELs for miosis, EEG effects, and visual evoked response (VER) were

evaluated at 7.5, 15, 25, 50, and 150 μg/m^3 (N=2 per group). Controls (N =6) were exposed to air for 5 h. For miosis, the LOAEL (5% decrement in pupil size compared with controls; estimated to be equivalent to approximately 10% decrement in pupil area; p<0.05) was reported to be 1.8±0.3 mg·min/m^3. The LOAEL (p<0.05) for changes in EEG parameters and VER was 0.8 mg·min/m^3 (indicative value). There was no significant decrease in blood AChE activity at any GB vapor exposure concentration tested.

Atchison et al. (2001) reported that subcutaneous injections of 0.4 LD_{50} GB once per day, 5 d/wk, for 13 wk in young male Hartley guinea pigs (600 g) resulted in no clinical signs of acute toxicity and no changes in body weight, body temperature, complete blood counts, or blood chemistry; however, RBC-ChE activity was decreased by about 90%. The subcutaneous LD_{50} for guinea pigs was reported to be 42 μg/kg.

Agent GD

Benschop et al. (1998) evaluated the toxicokinetics and effects of single inhalation exposures of the four stereoisomers of soman to guinea pigs. The test animals (male albino outbred guinea pigs of the Dunkin-Hartley type, 450–620 g body weight) were anesthetized and atropinized and then exposed, nose-only, for 5 h to each of the four stereoisomers, at a concentration of 20 ppb (160±16 μg/m^3). During the exposure there was a gradual increase in the inhibition of RBC-AChE, which correlated well with the increase in the concentration of the toxic stereoisomers (C(±)P(−) soman) in the blood. Inhibition of AChE in the brain and diaphragm was not significant at the end of the exposure period.

Atchison et al. (2001) reported that subcutaneous injections of 0.4 LD_{50} GD once per day, 5 d/wk, for 13k w in young male Hartley guinea pigs (600 g) resulted in no clinical signs of acute toxicity, no agent related pathology, and no change in blood chemistry other than a 91% inhibition of RBC-ChE. The subcutaneous LD_{50} for guinea pigs was reported to be 28 μg/kg.

Agent VX

Atchison et al. (2001) reported that subcutaneous injections of 0.2 LD_{50} VX once per day, 5 d/wk, for 13 wk in young male Hartley guinea pigs

(600 g) resulted in no clinical signs of acute toxicity and no changes in body weight, blood count, blood chemistry of gross, or histopathology; however, RBC-ChE activity was inhibited about 90%. The subcutaneous LD_{50} for guinea pigs was reported to be 9 μg/kg.

Crook et al. (1983) conducted VX vapor exposure studies in male and female Hartley guinea pigs. Crook and his colleagues consider their results to be nonverifiable and suspect for the reasons outlined earlier. These data are thus considered too unreliable for application to development of AEGL estimates for agent VX.

3.2.6. Rabbits

Callaway and Dirnhuber (1971) performed a study in which pupil area decrement was measured from electronic flash photographs of dark-adapted eyes for which baseline pupil area had been previously determined. The nominal cumulative exposure (Cts) necessary to produce 50% and 90% decrement in total pupil area were determined and compared for GB vapor (46 eye measurements from 14 rabbits), GD vapor (153 eye measurements from 48 rabbits), and T-2715 (GF analog) vapor (85 measurements on 19 rabbits). The cumulative exposure needed to produce miosis sufficient to generate 90% pupil area decrement was 2.71 mg·min/m^3 (95% CI=1.84– 4.00 mg·min/m^3) for agent GB, 2.19 mg·min/m^3 (95% CI=1.45–3.29 mg·min/m^3) for agent GD, and 1.79 mg·min/m^3 (95% CI=1.40–2.29 mg·min/m^3) for agent GF. The cumulative exposure needed to produce miosis sufficient to generate 50% pupil area decrement was 1.32 mg·min/m^3 (95% CI=1.05–1.67 mg·min/m^3) for agent GB, 0.59 mg·min/m^3 (95% CI=0.49–0.70 mg·min/m^3) for agent GD, and 0.75 mg·min/m^3 (95% CI=0.65–0.87 mg·min/m^3) for agent GF.

Callaway and Dirnhuber (1971) also evaluated the "miotogenic potency" of GB vapor in rabbits exposed to GB "under goggles" (43 miosis responses in 10 albino rabbits). The "goggle" experiments were designed to deliver GB vapor directly to the air volume around the eye and enclose the vapor as a means of controlling the exposure (no inhalation or percutaneous exposure) and delivering the vapor directly to the surface of the eye (thereby reducing variability). An airstream of GB vapor was delivered to the space enclosed by each goggle. The unexposed pupil area of each eye was considered to be the baseline for pupil area decrement determinations for each eye. Exposure periods ranged from 10 min to 5 h. Callaway and Dirnhuber (1971) reported a 50% decrement of pupil area in

the rabbit dark-adapted eye (goggles) at a Ct of 2.33 mg·min/m^3 (95% CI =1.65–3.31 mg·min/m3). A 90% decrement of pupil area occurred at a Ct of 7.68 mg·min/m^3 (95% CI=4.90–19.50 mg·min/m^3).

Agent VX

Crook et al. (1983) conducted VX vapor exposure studies in male and female New Zealand white rabbits. Crook and his colleagues consider their results to be nonverifiable and suspect for the reasons outlined earlier. These data are thus considered too unreliable for any application to development of AEGL estimates for agent VX.

In tests conducted by Goldman et al. (1988), blood cholinesterase levels were monitored in female rabbits (three per dose group) injected subcutaneously with VX at 0, 0.25, 1.0, 4.0, or 8.0 μg/kg once per day for 7 d. The 8.0 μg/kg dose was severely toxic (1/3 died, 2/3 ataxic). RBC-ChE activity was inhibited to 0.71 of the control value in the 0.25-μg/kg group, to 0.36 of the control value in the 1-μg/kg group, and to 0.24 of the control value in the 4.0-μg/kg group.

In a study of miotogenic potency, Callaway and Dirnhuber (1971) exposed the eyes of male and female "albino" rabbits (*N*=45; no strain identified; 94 observations) to concentrations of VX agent vapor ranging from approximately 0.5 μg/m^3 to 25 μg/m^3 for varying time periods (approximately 2–400 min). Pupil diameters were recorded only after attaining maximal decrease, and decrease in pupil area per Ct was expressed as a percentage of the original area of the same eye. Maximal pupil diameter decrease usually occurred at times >30 min postexposure. The "percentage decrease" data underwent probit transformation to derive Cts necessary to produce 50% and 90% decrease in pupil area in the dark-adapted eye. For comparison, experimental exposures to nerve agents GB and GD under a similar protocol were also performed by the authors (Callaway and Dirnhuber 1971). Their results are reported in Table 1–19 below.

3.2.7. Summary of Nonlethal Toxicity in Animals

The summary of animal toxicity data has focused on short-term, subchronic, or chronic exposures to agent GB (Table 1–20). Results of inhalation exposure studies are emphasized; however, some pertinent data for other exposure pathways are included.

TABLE 1–19 Miosis in Rabbits Following Vapor Exposure to Agents VX, GB, and GD

Agent	50% Pupil Area Decrease ($mg \cdot min/m^3$)	95% CI	90% Pupil Area Decrease ($mg \cdot min/m^3$)	95% CI	Slope *(b)*
VX	0.04	0.03–0.05	0.23	0.12–0.45	1.70
GB	1.32	1.05–1.67	2.71	1.84–4.00	4.11
GD	0.59	0.49–0.70	2.19	1.45–3.29	2.24

Source: Callaway and Dirnhuber 1971.

The miotogenesis studies of GB vapor exposure recently published by van Helden et al. (2001, 2002) (male and female marmosets and male guinea pigs) and Mioduszewski et al. (2002b) (male and female SD rats) were well conducted, employed modern protocols, and examined a range of exposure durations significant to the AEGL process. Mioduszewski et al. (2002b) is the critical study for deriving AEGL-1 values for agent GB; van Helden et al. (2001, 2002) is a secondary and supportive study.

3.3. Neurotoxicity

The G agents (GA [tabun], GB [sarin], GD [soman], and GF) and agent VX are toxic organophosphate ester derivatives of phosphonic acid. They are commonly termed "nerve" agents as a consequence of their potent anticholinesterase properties and subsequent adverse effects on both smooth and skeletal muscle function as well as the central and peripheral nervous system. Although the inhibition of cholinesterases within neuroeffector junctions or the effector itself is thought to be responsible for the major toxic effects of nerve agents, these compounds can apparently affect nerve impulse transmission by more direct processes as well (for example, direct effects on muscarinic receptors) (see Section 4.2).

As described in Section 3.2.3, Kassa et al. (2001) evaluated the neurotoxic effects of agent GB in male albino Wistar rats exposed for 60 min, once or repeatedly, to concentrations at 0.8, 1.25, or 2.5 mg/m^3. The lowest concentration was determined asymptomatic based on clinical and laboratory measurements. The second concentration was determined asymptomatic based on clinical signs, but produced a significant inhibition of RBC-AChE (30%). The highest test concentration was a nonconvulsive

TABLE 1–20 Nonlethal Toxicity of Agent GB Vapor to Animalsa

Species	Exposure	Duration	End Point	Comments	Reference
Dog	10.5 mg·min/m^3 20 min/d	2 mo	LOAEL	Miosis	Harris et al. 1953
Dog	15 mg·min/m^3 20 min/d	7–10 d[b]	LOAEL	Body tremors, dyspnea, loss of muscle control, convulsions	Harris et al. 1953
Dog	0.24–0.26 mg/m^3; 8, 16, 24 min/d	17–21 times over 4 wk	NOAEL	Nose only exposures; no reported toxic signs; ChE was not monitored	Fogleman et al. 1954
Dog	0.73–0.75 mg/m^3; 8, 16, 24 min/d	30 times over 6 wk	LOAEL	Dyspnea, gluteal muscle fasciculations in one of three test animals	Fogleman et al. 1954
Dog	2.38–2.43 mg/m^3; 8, 12, 16 min/d	30 times over 6 wk	LOAEL	Dyspnea; gluteal muscle fasciculations; RBC-ChE levels 0, 35%, and 35% of normal after 4 d	Fogleman et al. 1954
Dog	0.04 mg/m^3 4 h/d, 5 d/wk	6 mo	LOAEL	Decreased RBC-ACHE; dyspnea, salivation, rhinorrhea, miosis	Jacobson et al. 1959
Dog	0.001 mg/m^3 6 h/d, 5 d/wk	52 wk	NOAEL	Abnormal EKGs in some dogs; however, baseline measurements were not available for all the test animals	Weimer et al. 1979
Rabbit	1.32 mg·min/m^3	10 min to 5 h	ECt_{50}	50% miosis	Callaway and Dirnhuber 1971

Rabbit	2.71 mg·min/m^3	10 min to 5 h	ECt_{50}	90% miosis	Callaway and Dirnhuber 1971
Guinea pig	0.8 mg·min/m^3	5 h	LOAEL	EEG changes and visual evoked response	van Helden et al. 2001, 2002
Guinea pig	1.8 mg·min/m^3	5 h	LOAEL	Miosis	van Helden et al. 2001
Marmoset	0.2 mg·min/m^3	5 h	LOAEL	EEG changes	van Helden et al. 2001, 2002
Marmoset	2.5 mg·min/m^3	5 h	LOAEL	Miosis	van Helden et al. 2001, 2002
Marmoset	25 mg·min/m^3	5 h	LOAEL	Visual evoked responses	van Helden et al. 2001, 2002
Mouse	5 mg/m^3, 20 min/d	10 d	LOAEL	Muscular weakness of the limbs and slight ataxia; inhibition ($p < 0.001$) of NTE activity in the brain (59.2%), spinal cord (47.4%), and platelets (55.4%); focal axonal degeneration of spinal cord; blood AChE inhibited by 27.3% and brain AChE by 19.2%	Husain et al. 1993
Rat	0.068 mg/m^3 (female)	10 min	EC_{50}	Miosis	Mioduszewski et al. 2002b

Species	Exposure	Duration	End Point	Comments	Reference
Rat	0.020 mg/m^3 (female)	60 min	EC_{50}	Miosis	Mioduszewski et al. 2002b
Rat	0.012 mg/m^3 (female)	240 min	EC_{50}	Miosis	Mioduszewski et al. 2002b
Rat	0.087 mg/m^3 (male)	10 min	EC_{50}	Miosis	Mioduszewski et al. 2002b
Rat	0.030 mg/m^3 (male)	60 min	EC_{50}	Miosis	Mioduszewski et al. 2002b
Rat	0.024 mg/m^3 (male)	240 min	EC_{50}	Miosis	Mioduszewski et al. 2002b
Rat	0.8 mg/m^3	60 min	NOAEL	Asymptomatic	Kassa et al. 2001
Rat	1.25 mg/m^3	60 min	NOAEL	Asymptomatic but with significant inhibition of RBC-ChE	Kassa et al. 2001
Rat	2.5 mg/m^3	60 min	LOAEL	Changes in immune system and neurobehavioral effects	Kassa et al. 2001
Rat	0.4 mg/m^3 1 h/d	1 d	NOEL	No overt neurotoxicity (tremors) observed	Henderson et al. 2000, 2001, 2002
Rat	0.001 mg/m^3 6 h/d, 5 d/wk	24 wk	NOAEL	No observed inhibition of blood ChE	Weimer et al. 1979

Rat	0.001 mg/m^3 6 h/d, 5 d/wk	52 wk	NOAEL	No observed inhibition of blood ChE; tracheitis occurred in some animals (see text); atrophy of the seminiferous tubules was not considered to be agent-related	Weimer et al. 1979

[a]Experimental data.
[b]Following a 2-mo exposure to a Ct of 10.5 mg·min/m^3.

symptomatic exposure. Controls were exposed to pure air only. Three months following the exposure, the control and exposed animals (10 per test group) were evaluated for GB-induced effects using biochemical, hematological, neurophysiological, behavioral, and immunotoxicological methods. None of the exposed animals showed any clinical signs of intoxication 3 mo after exposure. Test animals exposed to GB at 0.8 mg/m^3 exhibited no neurotoxic effects after 3 mo, when monitored using a functional observatory battery (FOB) and a test of excitability of the CNS, on the basis of observation of convulsive activity after intraperitoneal administration of pentamethylenetetrazol. The only significant effect (p<0.05) observed in rats exposed once to GB at 1.25 mg/m^3 was an increase in stereotyped behavior. Effects observed in rats exposed three times at 1.25 mg/m^3 included a significant increase (p<0.05) in the excitability of the CNS, significant alterations of mobility score (p<0.01), gait disorder (p< 0.001) characterized by ataxia, and a significant increase in stereotyped behavior (p<0.001). Animals exposed once at 2.5 mg/m^3 exhibited significant changes in mobility score (p<0.01), activity (p<0.01), gait score (p <0.01), gait disorder (p<0.001), and stereotyped behavior (p<0.01).

3.4. Developmental and Reproductive Effects

Due to the limited database for evaluating developmental or reproductive effects of nerve agent vapor inhalation exposure, other exposure routes were also examined.

3.4.1. Rats

Agent GB

The reproductive and developmental toxicity of GB was evaluated in a pilot study in which Sprague-Dawley rats were exposed to GB vapors (Denk 1975). In one series of inhalation tests, male rats were exposed to GB at 0.1 or 1 μg/m^3 for 6 h/d, 5 d/wk, for 1, 2, 8, or 12 wk or 6, 9, or 12 mo and then mated to unexposed females. Nineteen days after mating, the females were sacrificed and examined for number of corpora lutea, deciduomata, number of fetal deaths, and number of live fetuses. Mated pairs of rats were also exposed to the same GB concentrations for 1, 2, or

3 wk or until the pups were whelped. The incidence of intrauterine deaths was recorded and all fetuses were examined for abnormalities. In a third series of tests, males and females were exposed to agent GB (sarin) for 10 mo and then mated. The F_1 generation was mated at 12 wk of age, as was the F_2 generation. The number and gender of offspring, number of preweaning deaths, number weaned, and pup weights at various ages were recorded. Denk (1975) reported reduced rates of whelping in the F_0 generation, but reduced whelping rates were also seen in the controls, and this effect was thought to be due to the age of the animals at mating (12 mo old). No other adverse effects with respect to dominant lethal mutations, reproductive performance, fetal toxicity, and teratogenesis were observed.

Oral exposure studies in laboratory animals indicate that developmental or reproductive effects are not likely, even at dose levels that are maternally toxic. LaBorde and Bates (1986) (see also LaBorde et al. [1996]) conducted developmental toxicity studies on agent GB type I and GB type II using CD rats. The test animals were dosed with 0, 100, 240, or 380 μg/kg orally on days 6–15 of gestation. Females were weighed on gestational day 0, gestational days 6–16, and before death on gestational day 20. The test animals were observed for clinical signs of toxicity. At sacrifice, gravid uteri were weighed and examined for number and status of implants (alive, resorbed, or dead). Individual fetal body weight and internal or external malformations were recorded. Maternal toxicity (evidenced by excessive salivation, ataxia, lacrimation) and mortality (8/29 for GB type I and 13/29 for GB type II) occurred in the high-dose group. There were no significant differences among treatment groups in the incidence of resorptions or in the average body weight of live fetuses per litter. The only fetal morphological anomaly was fetal hydroureter, which occurred at a rate of 5.2%, 1.9%, 5.3%, and 2.1% with GB type I; and 4%, 5%, 3.2%, and 0.5% with GB type II in the 0-, 100-, 240-, and 300-μg/kg dose groups, respectively. The observed effect was not dose related and was therefore considered a spontaneous variant. Skeletal and cartilage variants occurred between dose groups, but they were not statistically significant.

Agent GA

There are intraperitoneal and subcutaneous exposure studies of agent GA in which developmental and reproductive toxicity were studied in maternal CD rats (Bucci et al. 1993). In both studies, the LOAEL for ma

ternal toxicity (salivation, lacrimation, nasal discharge, diarrhea) was attained in the absence of fetal malformations or adverse effects on fetal implantations or fetal weight.

Agent GD

Developmental studies in maternal rats orally exposed to agent GD (soman) were reported by Bates et al. (1990); the protocol was the same as that employed in the GB oral exposure studies of LaBorde and Bates (1986) and LaBorde et al. (1996) reported earlier. At doses that produced significant maternal toxicity and mortality, there was no evidence of fetal toxicity or prenatal mortality as evidenced by postimplantation loss, average body weight of live fetuses per litter, or malformations (Bates et al. 1990).

Agent VX

In studies conducted by Schreider et al. (1984), pregnant rats were dosed with VX at 0.25, 1.0, or 4.0 μg/kg by subcutaneous injection on days 6–15 of gestation (doses higher than 4.0 μg/kg were expected to cause excessive deaths). The animals were sacrificed on day 20 of gestation. The examined fetuses showed no evidence of malformations. Fetal body weight, litter size, and gender ratio were within normal limits.

Goldman et al. (1988) administered VX by subcutaneous injection to Sprague-Dawley rats on days 6–15 of gestation. The administered doses were 0, 0.25, 1.0, or 4.0 μg/kg/d. Body weight, frequency of visceral and skeletal abnormalities, litter size, and gender ratios were evaluated. There was no statistical evidence that VX affected any of the parameters studied. Blood cholinesterase levels were not monitored.

In a modified dominant lethal study, Goldman et al. (1988) administered VX by subcutaneous injection to male and/or female Sprague-Dawley rats and observed the effects on various parameters including terminal body weight, testes weight, testicular histopathology, maternal weight, implantation sites, resorptions, and total corpora lutea. The test animals were dosed with VX at 0 (saline control), 0.25, 1.0, or 4 μg/kg/d for 10 wk. Triethylenemelamine was used as a positive control. Exposure to VX produced no significant changes in body or organ weights. VX had no adverse effects on pre-implantation losses as evaluated by number of im

plants, live fetuses, dead fetuses, and resorptions. Microscopic examination of the testes did not reveal any abnormalities that could be attributed to VX exposure.

In a three-generation study, male and female Sprague-Dawley rats were dosed by subcutaneous injection with VX at 0 (saline controls), 0.25, 1.0, or 4.0 μg/kg/d, 5 d/wk (Goldman et al. 1988). The F_0 generation (11– 12 males and 24 females per dose group) was dosed for about 105 d after which they were mated, and the dosing continued through gestation and weaning (total duration of dosing 21–25 wk). Dosing of the F_1 generation began after weaning and continued for approximately 126 d after which they were mated, and dosing continued through gestation and weaning (total duration 24–27 wk). Five males and five females of each dose group of the F_2 generation were sacrificed at weaning. The study included analysis of pup mortality in each of the generations, body and organ weight changes and hematological parameters in the F_0 generation, and histopathological examination of tissues (including nervous system, reproductive system, gastrointestinal tract, lung, liver, and kidney) of the F_1 parental males and females, the F_1 weanlings, and the F_2 weanlings. Blood cholinesterase activity levels were not monitored during the study. VX exposure had no adverse effect on the number of pups born in the F_1 or F_2 generation. Perinatal mortality (i.e., percent of pups born dead or dying within 24 h of birth) was not significantly different among dose levels for both generations; however, perinatal mortality in the high-dose group (5.7%) was considerably higher than that in the lower-dose groups (1.2%). Pup mortality from birth to weaning was significantly related ($p<0.01$) to VX exposure, primarily for the F_1 generation pups in the 4.0-μg/kg/d dose group. Goldman et al. (1988) attributed this increase to the effect of VX on the dams, which resulted in an increased cannibalism of the pups by the dams. The investigators concluded that under the conditions of the test, there was no evidence of direct VX reproductive toxicity. The hematological studies conducted on dosed males of the F_0 generation revealed no significant VX-associated effects. In females dosed with VX at 4.0 μg/kg/d, statistically significant decreases occurred in hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin. Body and organ weight analysis and histopathological examination revealed three effects that may have been dose-related changes in brain weight, incidence of eosinophilic gastritis, and incidence of pituitary cysts; however, Goldman et al. (1988) attributed the first two effects to statistical chance and considered the third not biologically significant. The overall conclu

sion of the investigators was that there were no organ-weight or microscopic changes that could be attributed specifically to the action of VX.

3.4.2. Guinea Pigs

Pregnant guinea pigs were administered GD orally (7 μg/kg/d) on gestation days 42, 43, and 44 (Mehl et al. 1994). It had been determined prior to the study that a dose of 13 μg/kg, while tolerated by nonpregnant females, was "highly toxic" to pregnant animals. The administered dose of GD caused no significant change in brain weight of neonates, the end point of concern, or in total body weight.

3.4.3. Rabbits

Agent GB

LaBorde and Bates (1986) (see also LaBorde et al. [1996]) conducted developmental toxicity studies on agent GB type I and GB type II using New Zealand rabbits. The same protocol as previously outlined for the rat oral studies by these same investigators (see Section 3.4.1) was employed in the rabbit study. The test animals were dosed with GB at 0, 5, 10, or 15 μg/kg orally on days 6–19 of gestation. No fetal toxicity or teratogenicity was observed. The only observed fetal anomaly was retinal folding, which occurred at a rate of 6.8%, 3.9 %, 4.3 %, and 7.4% for GB type I and 17%, 18%, 25%, and 19% for GB type II in the 0-, 5-, 10-, and 15-μg/kg dose groups, respectively. The frequency of the anomaly was not dose-related and was, therefore, considered to be a spontaneously occurring malformation. Maternal toxicity, evidenced by excessive salivation, ataxia, and lacrimation, occurred at the highest dose.

Agent GA

The developmental and reproductive toxicity of GA was studied in maternal New Zealand white rabbits dosed intraperitoneally or subcutaneously (Bucci et al. 1993). In both studies, the LOAEL for maternal toxicity

(salivation, lacrimation, nasal discharge, diarrhea) was attained in the absence of fetal malformations or adverse effects on fetal implantations or fetal weight.

Agent GD

Developmental studies in maternal rabbits orally exposed to agent GD (soman) were reported by Bates et al. (1990); the protocol was the same as that employed in the GB oral exposure studies of LaBorde and Bates (1986) and LaBorde et al. (1996) reported earlier. At doses that produced significant maternal toxicity and mortality, there was no evidence of fetal toxicity or prenatal mortality as evidenced by post-implantation loss, average body weight of live fetuses per litter, or malformations (Bates et al. 1990).

Agent VX

Goldman et al. (1988) administered subcutaneous doses of VX at 0, 0.25, 1.0, and 4.0 μg/kg/d to New Zealand white rabbits on days 6–19 of gestation. Animals were also observed daily for signs of toxicity. The does were sacrificed on day 29 of gestation. Body weight, fetal weights, fetal deaths, frequency of visceral and skeletal abnormalities, litter size, and gender ratios were evaluated. There was no statistical evidence that VX affected any of the parameters studied. Blood cholinesterase levels were monitored in a 7-d pilot study, which also included a dose of 8 μg/kg. The 8-μg/kg dose was severely toxic to the rabbits (1/3 died, 2/3 ataxic). The dose of 0.25 μg/kg resulted in a level of RBC-AChE inhibition equal to 0.71 of the control value, but produced no signs of toxicity.

3.4.4. Sheep

Agent VX

The effects of VX on the development and reproduction of sheep were evaluated by Van Kampen et al. (1970) following an accidental release of VX in Skull Valley, Utah. Of some 6,300 affected animals, about 4,500

died or were killed (Van Kampen et al. 1970). Seventy-nine surviving animals pregnant at the time of exposure, and their lambs, were evaluated for changes in RBC-AChE activity and for signs of toxicity over a 6-mo postexposure period. RBC-AChE activity in the ewes remained significantly depressed for about 4 mo and then returned to normal. Ewes that were sacrificed at 2-wk intervals had no gross or microscopic evidence of damage to the central nervous system. Torticollis (wryneck) developed in one ewe 1 wk following exposure and persisted for 9 mo. (Van Kampen et al. [1970] reported that a similar effect was seen in one of 38 ewes dosed in the laboratory with an undisclosed amount of VX.) Of the lambs born 2–3 mo after the exposure of the ewes, only one (total number examined not reported) exhibited a deformity (extra oral opening below the right ear), but Van Kampen et al. believed the anomaly originated developmentally and before the poisoning episode. None of the lambs displayed neurotoxic signs or symptoms, and their whole blood cholinesterase activity was not reduced even when suckling from exposed and affected ewes. Five months after exposure, the ewes exposed in the field as well as ewes dosed with an undisclosed amount of VX 4 mo prior were mated to unexposed males. Examination 4 mo later indicated that fetal growth and development were normal except for one fetus that appeared stunted (total number examined not reported). The investigators concluded that VX had little or no effect on fetal growth or development.

3.4.5. Summary

Animal data from vapor and oral exposure studies for agent GB suggest that agent GB does not induce reproductive or developmental effects in mammals. Oral exposure studies of agents GA and GD in laboratory animals as well as injection exposure studies of agent GA suggest the lack of reproductive or developmental effects for these agents. Available data indicate that agent VX does not cause reproductive or developmental effects.

3.5. Genotoxicity

Agent GB

In bioassays using bacteria and mammalian cell cultures, agent GB was

not genotoxic or mutagenic when tested with or without metabolic activation (Goldman et al. 1987). GB did not induce biologically significant increases in mutations (e.g., highest concentration tested failed to exceed a doubling of the spontaneous rate) when tested in the Ames *Salmonella* assay using five revertant strains (TA135, TA100, TA98, TA1537, and TA1538) (Goldman et al. 1987). GB type I and GB type II did not induce significant increases in forward mutations when tested on mouse L5178Y lymphoma cells at concentrations of 50, 100, or 200 μg/mL (Goldman et al. 1987). An increase in sister chromatid exchanges (SCE) was not observed in Chinese hamster ovary cells exposed in vitro to GB at 200 μg/mL (Goldman et al. 1987). Mice treated in vivo with a maximally tolerated intraperitoneal dose of GB at 360 μg/kg did not exhibit a significant increase in SCE in splenic lymphocytes (Goldman et al. 1987). Exposure of rat hepatocytes to GB concentrations as high as 2.4×10^{-3} M resulted in a decrease in DNA repair synthesis, leading Goldman et al. (1987) to conclude that GB probably did not damage DNA directly but that it might inhibit DNA synthesis after non-agent-induced DNA damage had occurred.

Agent GA

Genotoxicity and mutagenicity data for agent GA are available from microbial assays and in vitro and in vivo tests on laboratory animals (Wilson et al. 1994). GA was found to be weakly mutagenic in eight of 11 Ames *Salmonella* assays using the revertant strains TA98, TA100, TA1535, and TA1538 and S-9 activation. GA also induced dose-related increases in mutation rates when tested on mouse L5178Y lymphoma cells without metabolic activation; the increase observed at a test concentration of 100 μg/mL was nearly 3 times that of the control. An increase in sister chromatid exchanges (SCE) was observed in Chinese hamster ovary cells exposed in vitro to GA concentrations at 25–200 μg/mL. Dose-responses were linear and highly statistically significant; however, the number of SCEs did not exceed twice the control value at any of the concentrations tested. C57B1/6 mice treated in vivo with a maximally tolerated intraperitoneal dose of GA at 700 μg/kg did not exhibit a significant increase in SCE in splenic lymphocytes. Exposure of rat hepatocytes to GA concentrations as high as 200 μg/mL resulted in inhibition of unscheduled DNA synthesis. From the results of these studies (i.e., three positive responses in five assays), Wilson et al. (1994) concluded that GA was a weakly acting mutagen.

Agent VX

In tests on microorganisms and mammalian cell cultures, VX was not found to be mutagenic or was only weakly mutagenic (Crook et al. 1983; Goldman et al. 1988). Crook et al. (1983) reported that VX gave negative results when tested in the mouse micronucleus assay (exposures for 6 h/d for 9 d to VX at 0.002 mg/m^3) and when tested in the Ames assay with five strains of *Salmonella typhimurium* (compared with positive controls; no other data reported). VX did not induce biologically significant increases in mutations when tested in the Ames *Salmonella* assay using five revertant strains (TA135, TA100, TA98, TA1537, and TA1538) with and without metabolic activation (Goldman et al. 1988). In tests using the yeast *Saccharomyces cerevisiae,* VX did not induce recombinants following exposures to concentrations as high as 100 μg/mL (Goldman et al. 1988). VX also failed to induce forward mutations when tested on mouse L5178Y lymphoma cells at concentrations less than 50 μg/mL (Goldman et al. 1988). Although doses of VX at 50 and 100 μg/mL resulted in increased numbers of mutations; these were not more than 1.5 times the control level. (A 2-fold increase was considered the minimum required to establish a positive result.)

Crook et al. (1983) reported that VX gave negative results for mutagenicity when tested in the sex-linked recessive lethal assay using *Drosophila melanogaster*.

Summary

Agents GB and VX were not found to be genotoxic in a series of microbial, cellular and mammalian assays. Agent GA was reported to be weakly mutagenic in some microbial assays.

3.6. Carcinogenicity

Agent GB

As part of the chronic inhalation studies conducted by Weimer et al. (1979), the tissues of animals exposed to GB for up to 1 y were examined for microscopic lesions including tumors. The test species included ICR

Swiss mice, strain-A mice, Sprague-Dawley/Wistar rats, Fischer 344 rats, and purebred beagle dogs. The exposures were to GB at 0.0001 or 0.001 mg/m^3 6 h/d, 5 d/wk. Weimer et al. (1979) reported that agent-related tumors did not occur in any of the exposed species. Pulmonary tumors did occur in strain-A mice; after 52 wk of exposure, pulmonary adenomas were present in 3/19 animals exposed to GB at 0.0001 mg/m^3, in 3/20 animals exposed to GB at 0.001 mg/m^3, and in 0/20 controls. For animals maintained for 6 mo postexposure, the incidence rates for pulmonary adenocarcinomas were 5/19, 6/18, and 9/29, respectively. However, these lesions were not considered to be agent-related. Strain-A mice have a high natural propensity to form pulmonary tumors; the incidence of spontaneous pulmonary tumors being about 53% in animals 12 mo of age and 90% in animals 18 mo of age (Heston 1942). Overall, the studies of Weimer et al. (1979) indicate that agent GB is not carcinogenic.

Agent GA

No long-term animal carcinogenicity studies have been carried out on GA. Neoplastic lesions were not observed in male and female CD rats injected intraperitoneally with GA at up to 28.13, 56.25, or 112.5 μg/kg/d for 90 d (Bucci et al. 1992); however, this subchronic study was of insufficient duration to fully evaluate tumor incidence rates. No other animal data are available to assess the potential carcinogenicity of GA.

Agent VX

Standard long-term carcinogenicity studies have not been conducted on laboratory animals exposed to agent VX. Neoplastic lesions were not observed in male and female CD rats injected subcutaneously with 0.25, 1.0, or 4.0 μg/kg/d for 90 d (Goldman et al. 1988). No other animal data are available to assess the potential carcinogenicity of VX.

Summary

There is no evidence that agents GB, GA, or VX are carcinogenic. It is noted that a 90-d study, such as that performed by Bucci et al. (1992) for agent GA, is of insufficient duration to fully evaluate tumor incidence rates.

3.7. Summary

G Agents

Acute lethality data for inhalation exposures to the G agents are available in the form of LCt_{50} values for exposure times of 10 min or less. In only one published study was information presented from which a lethality threshold could be estimated for agent GD (Aas et al. 1985). Acute inhalation studies on rats exposed to agent GB vapor for the time periods of 10, 30, 90, 240, and 360 min have been conducted by the U.S. Army's Edgewood Chemical Biological Center (ECBC) at Aberdeen Proving Ground, Maryland (Mioduszewski et al. 2000, 2001, 2002a). Mioduszewski et al. (2000, 2001, 2002a) is the critical study for deriving AEGL-3 values for agent GB. Nonlethal toxicity studies conducted primarily on dogs indicate that low concentrations of the G agents may cause miosis, salivation, rhinorrhea, dyspnea, and muscle fasciculations. Studies on dogs and rats indicate that exposures to GB at 0.001 mg/m^3 for up to 6 h/d are unlikely to produce any signs of toxicity.

Animal data from vapor and oral exposure studies suggest that agent GB does not induce reproductive or developmental effects in mammals. Oral exposure studies of agents GB and GD in lab animals as well as injection exposure studies of agent GA suggest the lack of reproductive or development effects for these agents. Agent GB was not found to be genotoxic in a series of microbial and mammalian assays, but agent GA was reported to be weakly mutagenic. There is no evidence that agents GB and GA are carcinogenic.

Agent VX

Credible acute lethality data for vapor inhalation exposure to agent VX vapors are available for only two species (mice and goats) (Koon et al. 1960, as cited in NRC 1997). LCt_{50} values are 13.6 $mg \cdot min/m^3$ for mice and 9.2 $mg \cdot min/m^3$ for goats. In a short-term inhalation study, no signs of toxicity except miosis were seen in rats, mice, guinea pigs, or rabbits exposed to VX vapor concentrations at 0.0002 mg/m^3 or less (6 h/d, 5 d/wk, for 2 wk) (Crook et al. 1983).

The available data indicate that VX does not cause reproductive or developmental toxicity. There is no evidence suggesting that VX is genotoxic or carcinogenic.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism, Toxicokinetics, and Disposition

4.1.1. Absorption

Although nerve agents may be absorbed through any body surface, the route through which absorption is most rapid and complete is the respiratory tract. It has been reported that as much as 70% of an inhaled dose of agent GB is retained in guinea pigs, dogs, and monkeys (Oberst 1961). In studies conducted on human volunteers, Oberst et al. (1968) found that mean percent retention of an inhaled dose of agent GB ranged from about 80% to 90%. Resting men (minute volume 6.9–7.9 L/min) retained a similar percent of the inhaled dose regardless of whether they were breathing exclusively through the mouth or nose; however, exercising men (minute volume 42.5 L/min) retained a significantly lower percentage (80%). Toxicity studies on nonhuman primates indicate that the intravenous and inhalation dose levels producing a similar level of effect are about the same, also suggesting that absorption through the respiratory tract may be close to 100% of the inhaled dose (Johnson et al. 1988; Anzueto et al. 1990). However, in species such as rodents that are nasal breathers, a proportionally greater amount of toxicant may be removed before reaching the lungs (the mechanisms of removal are thought to be hydrolysis or a reaction with epithelial tissues). In guinea pigs, Allon et al. (1998) found that approximately 29% of an inhaled dose of a racemic mixture of agent GD (soman) reached the blood.

4.1.2. Toxicokinetics

Spruit et al. (2000) conducted toxicokinetic studies of (±) sarin in anesthetized, atropinized, restrained guinea pigs. The test animals were exposed (nose-only) to doses corresponding to 0.4 and 0.8 LCt_{50} for 8-min exposure times. Toxicokinetics was also studied after an intravenous bolus corresponding to 0.8 LD_{50}. The LC_{50} for sarin was calculated by probit analysis to be 47 mg/m^3 (95% CL=44–50 mg/m^3), and the LCt_{50} for an 8-min exposure was estimated to be 376 $mg \cdot min/m^3$. In both the intravenous and inhalation studies the concentration of the nontoxic (+) isomer in the blood was below detection limits (<5 pg/mL blood). In the intravenous test, the toxicokinetics of the toxic (−) isomer followed a bi-exponential

equation. In the inhalation tests, the blood AChE activity decreased to about 70% of control values at 0.4 LCt_{50} and to about 15% of control values at 0.8 LCt_{50}; however, there were no effects on respiratory parameters (respiratory minute volume or respiratory frequency). The toxic (−) isomer appeared to be rapidly absorbed and the toxicokinetics followed a discontinuous process with a mono-exponential equation for the exposure period and a bi-exponential equation for the postexposure period.

Benschop et al. (2000) (see also Benschop [1999]) studied the toxicokinetics of several VX stereoisomers [(±)−] in hairless guinea pigs (intravenous and percutaneous exposures) and marmosets (intravenous exposures only). Following an intravenous dose of 28 μg/kg (marmosets) or 56 μg/kg (guinea pigs), VX was found in the blood at toxicologically relevant levels even after 6 h. Detoxification proceeded at a slower rate in marmosets than in guinea pigs. The VX metabolite, O-ethyl methyphosphonic acid (EMPA), was found in the blood of the exposed animals; however, the metabolite contributed only 5% to the recovery of the phosphonyl moieties related to the VX dose. Metabolites of VX were also evaluated in in vitro studies by treating liver homogenates and plasma from hairless guinea pigs, marmosets, and humans with the radio-labeled compounds, ^{35}S-VX and [$^{14}CH_3$-P]-VX. The potential toxic metabolite VX-N-oxide was not found. Desethyl-VX was found after incubation of VX in plasma of all three species; however, because of its slow rate of formation, Benschop et al. (2000) concluded that it would be unlikely that this compound would be present at toxicologically relevant levels after administration of VX in vivo. In vitro studies with ^{35}S-VX revealed that a significant part of the thiol-containing leaving group (S-2-(N, N-diisopropylamino) ethane thiol, DPAT) was bound to proteins such as albumin. It was found that the sulfur-containing leaving group was also transformed into a variety of oxidation products.

4.1.3. Disposition and Metabolism

There are a number of enzyme systems in mammalian blood and tissues capable of the binding with and/or metabolically detoxifying organophosphate nerve agents. A primary disposition pathway is the binding of the compounds with blood cholinesterases and carboxylesterases. Of the cholinesterases present in blood (RBC- and plasma-ChE), VX preferentially inhibits RBC-ChE (Sidell and Groff 1974). Plasma cholinesterase may likely serve as a buffer to offset the binding of nerve agents (and pref

erential binding of agent VX) to RBC-AChE. It has been reported that pretreatment with human plasma cholinesterase protected laboratory rats (Ashani et al. 1993) and monkeys (Raveh et al. 1997) from lethal and other acute toxic effects of VX exposure.

The G-agents have a strong affinity for carboxylesterases (Jokanović 1989), in contrast to agent VX, which has a quaternary ammonium group that prevents it from being a substrate for carboxylesterases. In tests on male SD rats, Maxwell (1992) experimentally confirmed that endogenous carboxylesterases provide "significant protection" against in vivo toxicity of the organophosphorous (OP) agents GA, GB, and GD, but not VX. Maxwell (1992) goes on to conclude that "CaE [carboxylesterase] detoxification does not appear to be important" against exposures to lethal concentrations of agent VX in the laboratory rat.

Because of the lack of other reactive esterases, agent VX induces a toxic response at lower concentrations than the G agents.

While carboxylesterases were once widely considered to be absent from the blood plasma of humans, carboxylesterases are, indeed, present in human erythrocytes and monocytes as well as in human liver, kidney, lung, skin, and nasal tissue (Cashman et al. 1996). Additional literature documents the presence of carboxylesterases in many human tissues and fluids, including brain, milk, mammary gland, pancreas, small intestine, colon, stomach, placenta, and plasma and serum (Chanda et al. 2002; Kaliste-Korhonen et al. 1996). The lung carboxylesterases are associated with alveolar macrophages (Munger et al. 1991). Further, carboxylesterases are present in human tissues and organs where exposure to nerve agent vapors would likely first occur (nasal tissues and the lung), would be distributed (erythrocytes, monocytes, plasma), and would generate effects (brain, stomach, colon, etc.). Carboxylesterase is also present in human serum. Chanda et al. (2002) indicate that full characterization of the OP-protective capabilities of carboxylesterases requires assessment not only of the *amount* but also of the *affinity* exhibited by carboxylesterases for the inhibitor as well as the total carboxylesterase activity unlikely to be inhibited (inhibitor resistant esterase activity [IRE]). The detoxification potential of carboxylesterases is multifaceted and is an area requiring further experimental characterization.

It is acknowledged that the CaE profile in humans is not well known and that there are few data from which to characterize the contributions that CaE may make to human protection from anticholinesterase poisoning. Chanda et al. (2002) consider that full characterization of CaE amount, affinity, and IRE in human tissues will be necessary before accurate predic

tions can be made regarding CaE detoxification potential following anticholinesterase exposures to humans.

Phosphorylphosphatases associated with the hydrolysis of GD (somanase), GA (tabunase), and GB (sarinase) have been reported. Sterri et al. (1980) reported that the liver of rats was capable of hydrolyzing GD at a rate of 743 μmol/g of liver per hour. At low substrate concentrations some phosphorylphosphatases have been shown to be stereospecific in their activity. Sarinase from the plasma of rats preferentially catalyzes the hydrolysis of the less toxic isomer of agent GB (Christen and van den Muysenberg 1965); however, tabunase targets the toxic stereoisomer of agent GA (reviewed by Gupta et al. [1987]). Somanase from rat liver (Wahllander and Szinicz 1990) or swine kidney (Nordgren et al. 1984; Benschop et al. 1981) preferentially inhibits the less toxic isomers of agent GD; however, another hepatic enzyme in rat liver has been reported to be capable of hydrolyzing all four isomers of GD (Little et al. 1989). The same hepatic enzyme also catalyzed the hydrolysis of agents GA and GB (Little et al. 1989). In studies conducted on rats dosed subcutaneously with agent GB, GD, or GF at 75 μg/kg, Shih et al. (1994) found that the major metabolite formed by a nonsaturable mechanism and excreted in the urine was an alkylmethyl phosphonic acid. Little et al. (1986) identified ^{3}H-labeled GB metabolites in the tissues of mice following intravenous administration of a sublethal dose (80 μg/kg). Most of the label was associated with free isopropyl methylphosphonic acid (IMPA), the hydrolytic metabolite of GB. In individuals allegedly exposed to GB, Noort et al. (1998) found O-isopropyl methylphosphonic acid in serum samples, and Nakajima et al. (1998) reported that methylphosphonic acid and isopropyl methylphosphonic acid were detected as urinary metabolites of GB. Distribution of the low-sarinase allele appears to be somewhat ethnically related. The Japanese population has a higher frequency of the low-sarinase isoform (allele frequency of 0.66) than other ethnic groups (0.24 to 0.31) (Yamasaki et al. 1997).

A-esterases (paraoxonase/arylesterase) present in the blood and liver are also capable of hydrolyzing phosphate esters (Cashman et al. 1996). Paraoxonase is one A-esterase from humans known to hydrolyze the phosphorus-fluorine bond of the nerve agents GB and GD (Davies et al. 1996). A-somanase isolated from human liver (Wang et al. 1998) is capable of hydrolyzing agent GD as well as agent GA with P-F or P-CN bonding, but cannot hydrolyze paraoxon or nerve agent VX with P-O or P-S bonding. Agent GB was not tested in the studies of Wang et al. (1998). A-

esterases are considered to provide protection against the adverse effects of some OP compounds (Pond et al. 1995).

Paraoxonase is polymorphic in human populations, and individual differences are wide (LaDu et al. 1986, as cited in Davies et al. 1996; Furlong et al. 1988, 1989). In one population tested, differences in paraoxonase activity among three genotypes was approximately 6-fold (Kujiraoka et al. 2000). Among a "caucasoid" population sampled in Seattle, Washington, a 40-fold variation in human serum paraoxonase activity was observed (Furlong et al. 1989) and was associated with three phenotypes: homozygotes for the low-activity allele, heterozygotes, and homozygotes for the high-activity allele.

Individuals expressing certain isomeric forms of the enzyme with low hydrolyzing activity are considered to be more susceptible to organophosphate anticholinesterase poisoning (Yamasaki et al. 1997). The polymorphic paraoxonase gene (PON1) has an important role in the detoxifying metabolism of nerve agents and OP insecticides. The $PON1_{R192}$ paraoxonase isoform hydrolyzes agents sarin (GB) and soman (GD) slowly when compared with the $PON1_{Q192}$ isoform (Furlong et al. 2002; Davies et al. 1996). The human population can be organized into three PON1*192 genotypes: $PON1_{Q192}$ homozygotes; heterozygotes; and $PON1_{R192}$ homozygotes (Furlong et al. 2002; Allebrandt et al. 2002). Frequency distributions of the PON1*192 variants have been examined in ethnically diverse populations (Allebrandt et al. 2002). The allele expressing low activity for agent GB and agent GD hydrolysis ($PON1_{R192}$) is significantly more frequent in African Americans (sampled in Brazil and North America) and Asians (sampled in China, Japan, and Canada) than in individuals of Indo-European descent (sampled in East India, Turkey, Canada, Russia, Germany, North America, England, France, the Netherlands, and Brazil). Nevertheless, Furlong et al. (2002) point out that "genotyping alone provides no information about PON1 levels, which can vary up to 13-fold between individuals" (homozygous for the low-activity allele) (see also Furlong et al. [1989] and Davies et al. [1996]).

The serum paraoxonase activity ranges observed by Furlong et al. (1989) and discussed in Davies et al. (1996) illustrate the presence of human genetic variability in one of several metabolic detoxification systems that can denature certain G agents. It is understood, however, that mere determination of serum paraoxonase activity alone is not sufficient to characterize whole-organism susceptibility to anticholinesterase exposure. There are many other metabolic detoxification mechanisms that are also

simultaneously active (e.g., RBC-ChE, tissue carboxylesterases). Further experimentation will be necessary before 13-time or 40-time differences in human serum paraoxonase activity can be translated into quantitative differences in whole-organism susceptibility to anticholinesterase compounds.

Some investigators have previously considered that low levels of paraoxonase in newborns may contribute to the observed sensitivity of newborn rats to organophosphate insecticides (Benke and Murphy 1975; Burnett and Chambers 1994, as cited in Davies et al. 1996). A recent investigation (Chanda et al. 2002) presents in vitro and in vivo evidence that carboxylesterases "are critical for explaining age-related sensitivity" of rat pups to the OP insecticide chlorpyrifos. The presence of low carboxylesterase activity, however, does not sufficiently characterize the greater susceptibility of rat pups to neurotoxic effects of some OP insecticides (Chanda et al. 2002).

A novel mouse-liver enzyme, unrelated to the paraoxonases, has been found to hydrolyze agents GB and GD (Billecke et al. 1999) in an in vitro assay of soluble fraction extracts from commercially available frozen mouse livers.

4.1.4. Distribution and Excretion

Several studies have examined the tissue distribution and excretion of G agents and their metabolites following parenteral administration to rodents. In studies conducted on rats dosed subcutaneously with agent GB (sarin), agent GD (soman), or GF at 75 μg/kg, Shih et al. (1994) found that the major route of elimination for all three agents was urinary excretion. For GD, the lung was the major organ of accumulation. McPhail and Adie (1960) dosed rabbits with radio-labeled (^{32}P) GB and found the highest levels of radioactivity in the lungs and kidney. Kadar et al. (1985) injected mice intravenously with a LD_{50} dose of ^{3}H-labeled agent GD. High levels of radioactivity were found in the lung and skin at 5 min to 24 h after the injection, with very small amounts in the CNS. Considerable accumulation of the label occurred in the urine, gall bladder, and intestinal lumen, suggesting that these were the main pathways of excretion. Little et al. (1986) measured the distribution of ^{3}H-labeled agent GB (sarin) and sarin metabolites in the tissues of mice following intravenous administration of a sublethal dose (80 μg/kg). Within 1 min all tissues contained large amounts

of the label, of which less than 10% represented agent GB (sarin). High concentrations of the metabolite were found in the kidneys and lungs, and only trace amounts of ^{3}H-labeled agent GB (sarin) were found in the brain within 15 min. In a continuation of these studies, Little et al. (1988) evaluated the distribution of ^{3}H-labeled agent GD (soman) and agent GB (sarin) in the brain of mice following sublethal intravenous doses (25 μg/kg and 80 μg/kg). The nerve agents were distributed evenly throughout the brain tissue with the exception of a 2- to 5-fold greater concentration in the hypothalamus.

4.2. Mechanism of Toxicity

The acute toxicity of the nerve agents is considered to be initiated by inhibition of acetylcholinesterase (AChE), an enzyme responsible for deactivating the neurotransmitter acetylcholine at neuronal synapses and myoneural junctions. Nerve agents phosphorylate the enzyme, thereby preventing deactivation of acetylcholine. Although the inhibited cholinesterase can be reactivated by the process of dephosphorylation, that is not possible once the nerve agent-cholinesterase complex undergoes "aging," which is thought to happen because of a loss of an alkyl or alkoxy group. Agent GD ages very rapidly, with a $t_{1/2}$ (time required for 50% of the enzyme to become resistant to reactivation) of 1.3 min (Harris et al. 1978). The aging half-time for agent GA is 46 h, as calculated from a rate constant of 2.5×10^{-4} per minute (de Jong and Wolring 1978), and the $t_{1/2}$ for agent GB has been reported to be 5 h (Sidell and Groff 1974). In the latter case, approximately 5% of the GB-enzyme complex reactivated spontaneously. In contrast to the results of these latter studies, Grob and Harvey (1958) had earlier reported that both GA and GB combined with ChE almost irreversibly within 1 h when tested in vitro. The complex formed between ChE and agent VX does not age significantly (half-life about 48 h), and the rate of spontaneous reactivation in humans has been reported to be as fast as 1% per hour (Sidell and Groff 1974).

Although nerve agents exert toxic effects on the central and peripheral nervous system indirectly through AChE inhibition (Koelle 1976, 1981), nerve agents may also affect nerve impulse transmission by additional mechanisms at neuromuscular junctions (Somani et al. 1992) and at neurotransmitter receptor sites in the CNS. Rao et al. (1987) reported that VX caused an increase in acetylcholine release at neuromuscular junctions in

the frog by an interaction with the nicotinic acetylcholine receptor-ion channel complex. Aas et al. (1987) reported alterations in muscarinic receptors in rat bronchi and lung tissue after subacute inhalation exposures to agent GD. In the CNS, nerve agents may act directly on muscarinic, nicotinic, and glutamate receptors. Bakry et al. (1988) reported that nanomolar concentrations of agent GD affected muscarinic ACh receptors that have a high affinity for [^{3}H]-*cis*-methyldioxalane binding. Rocha et al. (1998, 1999) reported that, in cultured rat hippocampal neurons, VX at 0.01 nM reduced the evoked release of the neurotransmitters γ-aminobutyric acid (GABA) and reduced the amplitude of evoked GABAergic postsynaptic currents. VX concentrations >1 nM decreased the amplitude of evoked glutamatergic currents. In the presence of a Na^+ channel blocker, VX increased the frequency of GABA- and glutamate-mediated miniature postsynaptic currents, a Ca^+ dependent effect reported to be unrelated to cholinesterase inhibition (Rocha et al. 1999). Chebabo et al. (1999) reported that 0.3–1 nM of agent GB reduced the amplitude of GABA-mediated postsynaptic currents but had no effect on the amplitude of glutamatergic-mediated postsynaptic currents. The observed effect was thought to be due to the direct interaction of GB with muscarinic acetylcholine receptors present on presynaptic GABAergic neurons. Chebabo et al. (1999) suggest that the selective reduction in the action-potential-dependent release of GABA in the hippocampus might account for GB-induced seizures. Lallement et al. (1991a,b) had earlier suggested that GD-induced overstimulation of glutamatergic receptors contributed to maintenance of seizures. Although these data indicate that nerve agents may have direct effects on the nervous system unrelated to AChE inhibition, the in vitro data do not provide a means of relating electrophysiological alterations in rat hippocampal neurons or determining a dose conversion to the integrative end point of whole-body lethality. Neither do they allow qualitative/quantitative comparisons directly relevant to lethality. The results were obtained largely from single cells in isolation from whole organisms and systems, and extrapolation from observations on individual cells is not presently possible. At present, nM-induced amplitude changes in postsynaptic currents in rat hippocampal neurons in vitro cannot be correlated to dose levels resulting in multisystem failure and death such as are needed for AEGL estimation.

It should be further noted that the effects of nerve agents on GABAergic transmission in the CNS may have profound implications for behavioral effects in laboratory animals and humans and may also contrib

ute to the induction of convulsions at higher doses (Bakshi et al. 2000). Nevertheless, given the present undefined application of noncholinergic data to AEGL estimation, reliance on the primary assumption of anticholinesterase action is consistent with recognized opinion (Bakshi et al. 2000).

Recent studies with cholinesterase inhibitors such as galantamine, which affect neuronal nicotinic AChE receptors in a similar manner to that reported for VX, have shown that such compounds have therapeutic benefits for patients with mild to moderately severe Alzheimer's disease (Maelicke et al. 2001). As such, these compounds might be helpful in stabilizing behavior in such patients by improving memory and cognitive and daily function.

As pentavalent phosphorous-containing compounds, the G agents may also indirectly generate neurotoxic effects through a noncholinergic mechanism involving the kinase-mediated protein Ca^{2+}/calmodulin kinase II (Ca^{2+}/CaM kinase II) (de Wolff et al. 2002; Abou-Donia and Lapadula 1990). The Ca^{2+}/CaM kinase II protein becomes activated by OP-induced phosphorylation and reacts with the cytoskeletal proteins found in neurofilaments to produce axonal degeneration in the large-diameter tracts of the spinal cord.

It is also understood that OP compounds interact with detoxification enzymes such as the carboxylesterases and A-esterases and that the degree of such interaction may alter the magnitude and extent of the toxic cascade following AChE inhibition (Pope and Liu 2002). Recent studies indicate that full characterization of the OP-protective capabilities of carboxylesterases requires assessment not only of the *amount* but also of the *affinity* exhibited by carboxylesterases for the inhibitor as well as the total carboxylesterase activity unlikely to be inhibited (inhibitor resistant esterase activity [IRE]) (Chanda et al. 2002). The detoxification potential of carboxylesterases is multifaceted and is an area requiring further experimental characterization.

4.3. Relative Toxic Potency

Because of the sparse animal and human toxicity data for agents GA, GD, GF, and VX, AEGLs for those agents will necessarily be derived from the AEGLs for agent GB by a relative potency method. The database for the nerve agents as a group is considered reasonably complete in that there

exist (1) experimental data for multiple species, including humans; (2) documented nonlethal and lethal end points that follow an exposure-response curve; (3) a known mechanism of toxicity common to all the nerve agents with the all end points representing a response continuum to inhibition of cholinesterase activity; and (4) no uncertainties regarding other toxic end points such as reproductive or developmental effects or carcinogenicity.

Because the mechanism of action is the same for all the nerve agents, data uncertainty is reduced and target organ effects are expected to be identical and to differ only in magnitude. Thus, a comparative method of relative potency analysis from the more complete data set for agent GB is appropriate. This approach has been applied before, in the estimation of nerve agent exposure limits (Watson et al. 1992; Mioduszewski et al. 1998). The relative toxic potency of cholinesterase inhibitors can be expressed in several ways, based on in vitro or in vivo data.

4.3.1. In Vitro Potency

The in vitro potency can be measured by either the bimolecular rate constant (k_i, in M/min) for the reaction of the agent compound with the enzyme or by the molar concentration causing 50% inhibition of the enzyme (I_{50}) in vitro. The relationship between I_{50} and k_i for a fixed time *(t)* of incubation is expressed by the following equation (Eto 1974):

$$I_{50} = \frac{0.695}{t \times k_i}$$

As summarized by A.D. Little, Inc. (1985), k_i values for GB are in the range of 1×10^6 to 2×10^7 M^{-1}/min^{-1} for acetylcholinesterase in rat brain tissue, and 1×10^7 M^{-1}/min^{-1} for butyrylcholinesterase in human serum. Reported k_i values for agent GD are 3.7×10^7 M^{-1}/min^{-1} for acetylcholinesterase in rat brain tissue, and 1×10^7 M^{-1}/min^{-1} for butyrylcholinesterase in human serum (A.D. Little, Inc. 1985). More recently, Maxwell (1992) reported k_i values of 4.5 (±0.7)$\times10^6$ M^{-1}/min^{-1} for agent GA, 1.2 (±0.3) $\times10^7$ M^{-1}/min^{-1} for agent GB, and 3.6 (±0.5)$\times10^7$ M^{-1}/min^{-1} for agent GD in in vitro tests conducted on rat brain AChE.

I_{50} data for several G agents have been tabulated by Dacre (1984). The pI_{50} (negative log of the molar concentration causing 50% inhibition of

cholinesterase) was reported to be 8.4–8.6 for GA and 9.2 for GD (Dacre 1984; Holmstedt 1959). Grob and Harvey (1958) reported that the in vitro potency of GB (I_{50}=0.3×10^{-8} mol/L) was 5 times that for GA (I_{50}=1.5× 10^{-8} mol/L).

The k_i values for agent VX have been reported to be 1.4±0.3×10^{8} M/min, respectively, for in vitro tests conducted on rat brain AChE (Maxwell 1992). In comparison, Maxwell (1992) reported a k_i value of 1.2 ±0.3×10^{7} M/min for agent GB. The corresponding I_{50} values are 5.8×10^{-8} M for agent GB and 5.0×10^{-9} M for agent VX. The GB:VX ratio for the I_{50} values is 11.7, indicating that VX is nearly 12 times more potent than GB in inhibiting rat brain acetylcholinesterase in vitro. This comparison is one way to express the relative potency of agent VX.

4.3.2. In Vivo Potency

Relative potency of nerve agents can also be expressed in terms of the in vivo dose necessary to produce the same toxic effect by a specific exposure route.

G Agents

A summary of the estimated inhalation and visual effects values for the G agents is given in Tables 1–21 and 1–22. The information presented on animal toxicity values is derived from Callaway and Dirnhuber (1971) and Mioduszewski et al. (2002b) for nonlethal visual effects; and Oberst (1961), Callaway and Blackburn (1954), Mioduszewski et al. (2001, 2002a), and Anthony et al. (2002) for lethality. Another source is the largely unpublished experimental data summarized by the NDRC in 1946.

Estimates of lethality and severe effect levels in humans are based on extrapolations from animal data and on modeling studies. Several of the estimates are presented in Table 1–21, together with the limited human data for miosis. For the end point of miosis, ratios for ECt_{50}, ECt_{90}, and threshold effects are summarized in Table 1–21 for both experimentally derived and estimated toxicity values. For miosis as a critical effect, comparison of effective doses to achieve 50% pupil area decrement in the eye of the albino rabbit (Callaway and Dirnhuber 1971) indicates that agents GD and GF are more miotogenic than GB at approximately 50% of the GB Ct (GB/ GD of 2.24; GB/GF of 1.76; relative potency to agent GB of approximately

TABLE 1–21 Comparison of Visual Effects Values for G Agents

	Toxicity value (ECt [mg·min/m^3])				Ratios				
Species	GB	GA	GD	GF	GB:GA	GB:GD	GB:GF	GD:GF	References
Human (10 min to 5 h) (ECt_{90}, miosis)	13.85		Callaway and Dirnhuber 1971						
Human (20 min) (ECt_{50}, miosis)	4		Johns 1952						
Human (ECt_{50}, incapacitation)	2.5	7.5	0.4		0.33	6.25			Wells et al. 1993[a]
Human (10 min to 5 h) (ECt_{50}, miosis)	2.33		Callaway and Dirnhuber 1971						
Human (20 min) (No effect, miosis)	1.2		McKee and Woolcott 1949						
Human (2 min) (ECt_{50}, mild effects)	0.5	0.5	0.2	0.2	1.0	2.5	2.5	1.0	Reutter and Wade 1994[a] (unclassified summary table)
Human (2 min) (ECt_{50}, mild effects)	0.5	0.5	0.25	0.25	1.0	2.0	2.0	1.0	Mioduszewski et al. 1998[a]
Human (2–10 min) (ECt_{50}, mild effects)	<2		NRC 1997[a]						
Human (1 min) (<ECt_{01}, miosis)	0.5		McNamara and Leitnaker 1971[a]						

Rat (f, m) (10 min) (ECt_{50} miosis)	0.68, 0.87						Mioduszewski et al. 2002b
Rat (f, m) (60 min) (ECt_{50}, miosis)	1.20, 1.80						Mioduszewski et al. 2002b
Rat (f, m) (240 min) (ECt_{50}, miosis)	2.88, 5.76						Mioduszewski et al. 2002b
Guinea pig (5 h) (LOAEL, miosis)	1.8						van Helden et al. 2001, 2002
Marmoset (5 h) (LOAEL, miosis)	2.5						van Helden et al. 2001, 2002
Rabbit (10 min to 5 h) (ECt, 50% miosis)	1.32	0.59	0.75[b]	2.24	1.76	0.79	Callaway and Dirnhuber 1971
Rabbit (10 min to 5 h) (ECt, 90% miosis)	2.71	2.19	1.79[b]	1.23	1.51	1.22	Callaway and Dirnhuber 1971

[a]Secondary sources.
[b]Data for agent T2715, (2-methylcyclohexyl methylphosphonfluoridate), analog for agent GF.

TABLE 1–22 Acute Lethal Inhalation Toxicity Values for G-Agents

Species (Exposure Time)	Toxicity Value (LCt_{50} [mg·min/m^3])				Ratios				
	GB	GA	GD	GF	GB:GA	GB:GD	GB:GF	GD:GF	Reference
Monkey	74	187			0.40				DA 1974[a]
Monkey (2 min)	42	135			0.31				Oberst 1961; DA 1974
Monkey (10 min)	150	250 180			0.71[b]				NDRC 1946[a,c]
Geometric Mean (monkey data)					0.44				
Rat (female) (1-min)	118		135	110		0.87	1.07	1.23	Callaway and Blackburn 1954
Rat (male) (1-min)	220		196	181		1.12	1.22	1.08	Callaway and Blackburn 1954
Rat (10-min)	220	450	230 279		0.49	0.87[d]			DA 1974[a]
Rat (female) (10-min; 24-h lethality)	184			253			0.73		Mioduszewski et al. 2001; Anthony et al. 2002
Rat (female) (60-min; 24-h lethality)	387			334			1.16		Mioduszewski et al. 2001; Anthony et al. 2002

Rat (female) (240-min; 24-h lethality)	741			533			1.39		Mioduszewski et al. 2001; Anthony et al. 2002
Rat (male) (10-min; 24-h lethality)	231	368	0.63	Mioduszewski et al. 2001; Anthony et al. 2002					
Rat (male) (60-min; 24-h lethality)	459	396	1.16	Mioduszewski et al. 2001; Anthony et al. 2002					
Rat (male) (240-min; 24-h lethality)	1,040	595	1.75	Mioduszewski et al. 2001; Anthony et al. 2002					
Geometric Mean (rat data)					0.49	0.95	1.09	1.15	
Overall Geometric Mean (rat and monkey)					0.47	0.95	1.09	1.15	

[a]Secondary sources.
[b]Based on geometric mean of 212 mg·min/m^3 for the two data points for GA.
[c]Summary of largely unpublished experimental data.
[d]Based on geometric mean of 253 mg·min/m^3 for the two data points for GD.

2) and that the GD:GF ratio approximates 1.0 (equal to 0.79). For 90% pupil area decrement in the rabbit, agents GD and GF are again more effective than agent GB for inducing this end point (GB/GD of 1.23, GB/GF of 1.51; relative potency range to agent GB of approximately 1.2 to 1.5) with a GD:GF ratio of 1.22. A protective determination of relative potency to agent GB is 2.0. Thus, agents GD and GF are considered equipotent and approximately twice as potent as agent GB for inducing miosis. For more severe effects, such as lethality, resulting from vapor exposures, the relative potency estimates presented in Table 1–22 indicate that agents GB, GD, and GF are equally potent and are twice as potent as agent GA.

At a public hearing in 2000 convened by the Chemical Demilitarization Branch of the Centers for Disease Control and Prevention, a U.S. Surgeon General's review panel concluded that because (1) the data base for GB is relatively robust, and (2) the data for the other G agents are limited, it is appropriate to utilize a relative potency approach for comparing G agents (67 Fed. Reg. 895 [2002]; DHHS 2002).

Agent VX

The in vivo doses of agents VX and GB required to produce the same level of blood cholinesterase inhibition in the same species by a specific exposure route are shown in Table 1–23. In humans, the experimentally determined RBC-AChE$_{50}$ for VX is 0.0023 mg/kg for an oral dose (Sidell and Groff 1974). In contrast, for agent GB, an oral dose of 0.010 mg/kg is required to produce about the same level of effect (Grob and Harvey 1958). The GB:VX ratio for this effect is approximately 4.3. In studies conducted by Gupta et al. (1991) in which rats were injected subcutaneously, VX was found to be 10 times more toxic than GB for ChE inhibition and myonecrosis end points. The relative potency of agents VX and GB are shown in Table 1–23.

In studies conducted by Maxwell (1992) on Sprague-Dawley rats, subcutaneous LD$_{50}$ values for GB and VX were 0.51 and 0.027 μmol/kg, respectively, indicating that VX is about 19 times more toxic than GB in rats for subcutaneous lethality, on a molar basis (if the micromoles of each compound are converted to grams using 140 as the molecular weight of GB and$\times 10^{-5}$ g/kg for GB and 8.022×10^{-5} g/kg for VX, resulting in a GB:VX ratio of 9.9). Analysis of parenteral data for Hartley albino guinea pigs (subcutaneous) and Swiss ICR mice (intramuscular) in studies by Koplovitz

TABLE 1–23 Relative Potency Estimates for Agents GB and VX Experimental Data

Species	Toxicity End Point	Units	GB	VX	GB:VX Ratio
Human	Inhalation Ct ChE_{50}[a]	mg-min/m^3	42	~6.5	~6.5
Human	Oral RBC-ChE_{50}[b]	μg/kg	10	2.4	4.3
Human	Intra-arterial/intravenous RBC-ChE_{50}[b]	μg/kg	3	1.1	2.7
Monkey[l]	Intravenous LD_{50}[c]	μg/kg	20	6–11.9	1.8–3.3
Rat[l]	Intravenous LD_{50}[c,d]	μg/kg	45–63	6.9–10	4.5–9.1
Mouse	Intramuscular LD_{50}[e]	μg/kg	204.81	13.07	15.7
Mouse[l]	Intravenous LD_{50}[c]	μg/kg	100	12–15	6.7–8.3
Mouse[l]	10-min LCt_{50}[c,d]	mg·min/m^3	240–310	4–13	18.5–77.5
Mouse[l]	Percutaneous LD_{50}[c,f]	μg/kg	1000	36–59	17–28
Guinea pig	Subcutaneous LD_{50}[e]	μg/kg	41.26	6.89	5.99
Rat	Subcutaneous LD_{50}[g]	μmol/kg	0.57	0.03	19 (9.9)[k]
Rat[l]	Oral LD_{50}[c]	μg/kg	870–1,060	77–128	6.8–13.8
Rabbit[l]	Percutaneous LCt_{50}[c,h]	mg·min/m^3	2,000	8.3–28	71–241
Rabbit	Vapor exposure; 50% pupil area decrement[i]	mg·min/m^3	1.32	0.04	33
Rabbit	Vapor exposure; 90% pupil area decrement[i]	mg·min/m^3	2.71	0.23	11.8

[a]GB, Oberst et al. (1968); VX, Bramwell et al. (1963) (estimated from tabulated data; not verifiable).

[b]GB, Grob and Harvey (1958); VX, Sidell and Groff (1974).
[c]DA (1974) (secondary source; not verifiable).
[d]Dacre (1984) (secondary source; not verifiable).
[e]Koplovitz et al. (1992)
[f]Liquid exposures.
[g]Maxwell (1992).
[h]Vapor exposures.
[i]Callaway and Dirnhuber (1971).
[k]Ratio shown in parenthesis based on grams per kilogram.
[l]Secondary source data.

et al. (1992) resulted in acute (24-h) LD_{50} estimates as follows: in the guinea pig, LD_{50} for GB of 41.26 μg/kg, LD_{50} for VX of 6.89 μg/kg; and in the mouse, LD_{50} for GB of 204.81 μg/kg, LD_{50} for VX of 13.07 μg/kg. Inhalation lethality data for mice include LCt_{50} values of GB at 240 mg·min/m^3 (forced activity); GB at 310 mg·min/m^3 (resting animals); VX at 4 mg·min/m^3 (total animal exposures); and VX at 13.6 mg·min/m^3 (head-only exposures) (DA 1974; Koon et al. 1960, as cited in NRC 1997).

The Cts necessary to generate 50% and 90% decrease in pupil area in the albino rabbit eye (Callaway and Dirnhuber 1971) were summarized in Table 1–19. The calculated Cts for 50% decrease are 1.32 mg·min/m^3 for GB and 0.04 mg·min/m^3 for VX (a GB:VX ratio of 33), while the Cts for 90% decrease are 2.71 mg·min/m^3 for GB and 0.23 mg·min/m^3 for VX (a GB:VX ratio of 11.8) (see Table 1–23). Callaway and Dirnhuber (1971) consider the 90% decrement to be a more definite end point; furthermore, this degree of pupil area decrease has operational significance. However, because Callaway and Dirnhuber (1971) do not document incidence data, neither an EC_{50} nor an EC_{90} for a given percentage miosis, as defined by current experimental protocols, can be reliably determined for their exposed rabbit population.

Primary experimental data for GB:VX comparisons for the same end point are available for five mammalian species (human, rat, mouse, guinea pig, rabbit; see Table 1–23). In all cases, agent VX is more potent than agent GB (range of 2.7 to 33).

Human Estimates ofGB and VX Toxicity

Estimates of lethality and severe effect levels in humans are based on extrapolations from animal data and on modeling studies. Several of these estimates are presented in Table 1–24, together with the limited human experimental data (vapor inhalation, oral, intra-arterial, and intravenous exposures) for ChE_{50} levels. The GB:VX ratios for these experimentally derived end points fall in the range of 2.7 to 6.5. For the end point of miosis, ECt_{50} estimates range from 0.06 mg·min/m^3 to 0.09 mg·min/m^3 for VX and 0.5 mg·min/m^3 to 1.5 mg·min/m^3 for GB, resulting in overall GB:VX ratios of 5.6 to 25 (secondary sources and nonverifiable data).

Human inhalation exposures (Oberst et al. 1968; Bramwell et al. 1963), human oral exposures (Grob and Harvey 1958; Sidell and Groff 1974), and human intra-arterial and intravenous exposures (Grob and Harvey 1958;

TABLE 1–24 Human Toxicity Estimates for Agents GB and VX

Toxicity End Point (Exposure Time)	GB (mg·min/m^3)	VX (mg·min/m^3)	GB:VX Ratio
Inhalation ChE_{50}[a]	42[b]	~6.5[c]	6.5
Oral RBC-ChE_{50}[a]	10 μg/kg[d]	2.3 μg/kg[e]	4.3
Intra-arterial/intravenous RBC-ChE_{50}[a]	3 μg/kg[d]	1.1 μg/kg[e]	2.7
LCt_{50} (2–10 min)	35[f]	15[f]	2.3
ECt_{50} (2–10 min)	25[f]	10[f]	2.5
LCt_{05}	20[g]	6[g]	3.3
ECt_{05} (severe)	1[g]	5[g]	3
LCt_{01}	10[d]	–	–
ECt_{05} (mild)	8[g]	3[g]	2.7
No deaths	6[h]	–	–
ECt_{05} (ocular, miosis)	1.5[g]	0.06[g]	25
Ocular threshold	1.0[i]	0.04[i]	25
ECt_{50} (ocular, miosis; 2–10 min)	0.5[f]	0.09[f]	5.6
ECt_{50} (ocular, miosis)	>0.5[k]	0.09[k]	>5.6

[a]Experimental data with human subjects; all other estimates are extrapolations based on animal data.
[b]Oberst et al. (1968); resting men, breathing 7 L/min.
[c]Bramwell et al. (1963); estimated from tabulated data—not verifiable.
[d]Grob and Harvey (1958).
[e]Sidell and Groff (1974).

[f]Reutter and Wade (1994).
[g]Wells et al. (1993).
[h]DA (1987).
[i]DA (1990b).
[k]NRC (1997).

Sidell and Groff 1974) are included in the experimental database summarized in Tables 1–23 and 1–24; reported end points for each human study were ChE_{50}. The Bramwell et al. (1963) study of VX inhalation toxicity is considered a flawed and nonverifiable source because the human subjects were not exposed to a rigorously controlled atmosphere (breathing zone concentrations could not be determined and potential effects of the carrier solvent [benzene] on agent absorption by subject was not evaluated, etc.). In consequence, the GB:VX ratio for inhalation ChE_{50} (which includes the VX Ct from Bramwell et al. [1963]) is not as credible as the comparable ratio derived from the well-conducted human oral exposure studies of Grob and Harvey (1958) and Sidell and Groff (1974).

4.3.3. Comparison of Exposure Standards

G-Series Agents

The current occupational exposure limits for the G-series nerve agents, as published by the CDC (DHHS 1988) are 0.0001 mg/m^3 for GB and GA to be applied as a no-adverse-health-effect level for 8-h continuous workplace exposure. The resulting GB:GA ratio is 1.0. The current general population exposure limits for the G-series nerve agents, as published by the CDC (DHHS 1988) are 0.000003 mg/m^3 for GB and GA, to be applied as a no-adverse-health-effect level for 24-h continuous exposure (provides a GB:GA ratio of 1.0). Agents GD and GF are not part of the unitary stockpile and were not evaluated by the CDC in 1988.

The U.S. Department of the Army has prepared a health criteria document for the G-series agents (Mioduszewski et al. 1998) in which exposure limits for the G agents were derived using the relative potency approach and the currently accepted exposure limits for agent GB. As part of a regularly scheduled review process, the CDC is currently reevaluating the 1988 agent control limits with application of recent risk assessment models and updated scientific data (67 Fed. Reg. 895 [2002]; DHHS 2002). The review is currently (September 2002) in progress, and the CDC has not yet released a final position.

Agent VX

The current occupational exposure limit for VX, as published by the

CDC (DHHS 1988), is 0.00001 mg/m^3. Compared with the CDC recommended value of 0.0001 mg/m^3 for agent GB, the resulting GB:VX ratio is 10. The current general population exposure limits for VX and GB, as published by the CDC (DHHS 1988), are 0.000003 mg/m^3 for GB and 0.000003 mg/m^3 for VX, resulting in a GB:VX ratio of 1.

The U.S. Department of the Army has prepared a health criteria document for VX (Reutter et al. 2000) in which exposure limits for VX are derived using the relative potency approach and the currently accepted exposure limits for agent GB (see Mioduszewski et al. [1998] and DHHS [1988], as detailed above). The exposure limits developed by Reutter et al. (2000) were based on minimal effect levels, and miosis was considered to be the most appropriate end point to use for comparison. Reutter et al. (2000) consider a ratio of 10 to be a protective estimate of the relative potency of miosis for agents GB and VX.

Embedded within the Army's logic (USACHPPM 1998; Reutter et al. 2000) regarding the choice of 10 for the relative potency of VX:GB are two elements: downward adjustment to allow for the greater effect of VX on the eye, and upward adjustment to allow for the more rapid recovery (reversibility) of eye effects from VX exposure compared with recovery following GB exposure. These adjustments are based on the human intravenous studies of Kimura et al. (1960) and a calculational model based on ChE activity recovery (McNamara et al. 1973). Ocular exposure to VX vapor is estimated to cause eye effects at approximately one-twenty-fifth of the GB Ct required to attain the same effect. VX "ages" (irreversibly binds to cholinesterase) very slowly ($t_{1/2}$ of 48 h) when compared with agent GB ($t_{1/2}$ of 5 h) (Sidell and Groff 1974), and some spontaneous enzyme recovery occurs even in the absence of antidote. In general, recovery from the effects of VX vapor exposure is 4 times greater than that for agent GB (McNamara et al. 1973). Thus, an effective concentration of VX relative to GB is four-twenty-fifths, or 0.16, or approximately one-sixth. The ratio of 1:10 used by the Army in deriving exposure criteria for VX (Reutter et al. 2000) was to allow for a greater margin of safety.

4.3.4. Selection of Nerve Agent Potency Values for Use in Deriving AEGLs

Recent publication of science policy by the EPA Office of Pesticides to guide the use and application of data on cholinesterase inhibition (EPA 2000) recommends a weight-of-evidence approach for evaluating toxicity

end points for anticholinesterase compounds. This approach is consistent with that of Storm et al. (2000), who consider that the most defensible means of deriving (occupational) inhalation exposure limits for organophosphates should be based on weight-of-evidence. In the weight-of-evidence approach, first priority is given to clinical signs and physiological or behavioral effects in humans and animals followed by

- Symptoms in humans.
- Cental nervous system acetylcholinesterase inhibition.
- Peripheral nervous system acetylcholinesterase inhibition.
- Red blood cell acetylcholinesterase inhibition.
- Plasma cholinesterase inhibition in humans and animals.

In general, the guidelines consider blood ChE inhibition to be an imperfect measure because of the need for individual baseline measurements for comparison and the fact that there is no fixed percentage of blood ChE activity change that can distinguish adverse from nonadverse effects (EPA 2000; Storm et al. 2000). A number of nerve agent exposure investigations have noted the poor association between blood (RBC and plasma) cholinesterase activity and anticholinesterase intoxication (Koelle 1994; Sidell 1992; Rubin and Goldberg 1957; Mioduszewski et al. 2002b). Circulating ChE activity does not parallel tissue ChE activity, and minimal blood ChE activity has been observed in association with normal tissue function (Sidell 1992). In the recent GB vapor exposure study of Mioduszewski et al. (2002b), “miosis was not correlated with, or even accompanied by, significant reduction of circulating AChE, BuChE, or CaE” as a consequence of GB vapor whole-body exposure to SD rats. These results further document the fact that miosis alone, and in the absence of signs such as ChE or CaE activity inhibition, is a local effect and reflects an exposure much less than that required to produce a systemic clinical effect. Thus, selection of the local effect of miosis as a critical AEGL end point allows a greater margin of protection against the potential for exposures that would generate systemic effects.

The findings of Mioduszewski et al. (2002b) are consistent with those for human volunteers exposed to GB vapor in the study of Rubin and Goldberg (1957).

Although RBC-ChE inhibition in the blood is an acceptable surrogate for central nervous system inhibition, plasma ChE is more labile and is considered a less reliable reflection of enzyme activity change at neuro

effector sites (EPA 2000; Young et al. 1999; California Environmental Protection Agency 1998). In consequence, plasma-ChE activity inhibition is considered a biomarker of effect and is here rejected as a critical end point from which to develop a reliable estimate of relative potency. Relative RBC-AChE inhibition or an observable sign (i.e., miosis) in a test species are considered more appropriate end points for deriving relative potency estimates.

G Agents

Experimental determination of miosis (90% decrease in pupil area) in the eyes of albino rabbits directly exposed to a range of GB, GD, and GF vapor concentrations for periods of time ranging from 2–10 min to 5–6 hours (Callaway and Dirnhuber 1971) is a suitable study for estimating relative potency between the G-series nerve agents.

Although there are acknowledged analytical weaknesses in the protocol and data of Callaway and Dirnhuber (1971), this experiment is the only study found in the literature for which the same end point is measured in the same species following exposure to each of several G-series agents. There are no comparable human experimental data. The resulting potency ratios, estimated from cumulative exposure (Ct) values in the literature, are presented in Table 1–21.

For AEGL-1 and AEGL-2 effects, GB and GA are considered equipotent, and GD and GF are each considered equipotent to each other, and more potent than GB by a factor of 2.0 for miosis (see Table 1–22 and the review by Mioduszewski et al. [1998]). Thus, for an equivalent effective concentration (EC) for miosis

EC of GA (mg/m^3)=EC of GB (mg/m^3);
EC of GD (mg/m^3)=EC of GB (mg/m^3)÷2; and
EC of GF (mg/m^3)=EC of GB (mg/m^3)÷2.

For AEGL-3 effects, GB, GD, and GF are considered equipotent, while GA is considered less potent than agent GB by a factor of 2 (see Table 1–22 and the review by Mioduszewski et al. [1998]). As previously discussed in Section 3.1.3, a secondary and short-term GD vapor inhalation study of rat lethality was performed for GD dynamic chamber exposure times of ≤30 min (Aas et al. 1985). In addition, a recent study of GF vapor inhalation

lethality in male and female SD rats reported 24-h LC_{50} and LCt_{50} values for 3 durations of exposure (10, 60, and 240 min) (Anthony et al. 2002). The assumptions for agent GD and GF lethal potency relative to agent GB is generally supported by analysis of the Aas et al. (1985) and Anthony et al. (2002) rat lethality data. Thus, for lethal concentrations (LC) of the G agents

LC of GB (mg/m^3)=LC of GD (mg/m^3)=LC of GF (mg/m^3); and
LC of GA (mg/m^3)=LC of GB (mg/m^3)×2.

Agent VX

The AEGL standing operating procedures (NRC 2001) state the following: "It is important to emphasize that only toxicity data obtained directly from a primary reference source is used as the basis for 'key' toxicity studies from which the AEGL values are derived. Additionally, all supporting data and information important to the derivations of an AEGL value is obtained solely from the primary references." In the studies listed in Tables 1–23 and 1–24, the verifiable experimental data for humans, rats, and rabbits provide a range of VX:GB relative potencies (RPs) of 2.7 to 33.

Of the various animal data available for developing a GB:VX relative potency factor, the rabbit miosis study of Callaway and Dirnhuber (1971) offers advantages in that VX and GB vapor were tested by the same investigators using the same protocols and test species (see Table 1–23). Nevertheless, it is understood that agent measurements collected during the study were hampered by the limited capabilities and techniques for determining agent vapor concentrations in the early 1970s. Furthermore, when compared with current low-light digital methods, the protocols employed to measure rabbit miosis in Callaway and Dirnhuber (1971) are today considered semisubjective. In addition, the study documentation does not fully report miosis incidence in the agent-exposed rabbit population.

When making cross-compound comparisons for use in developing human exposure guidelines, there is a preference for human data sets. Three exposure routes have been examined in the analysis presented in Tables 1–23 and 1–24. Remarkable concordance (RP range of 2.7 to 6.5) is noted.

The flawed and nonverifiable study of Bramwell et al. (1963) was described in previous sections. The GB:VX ratio for inhalation ChE_{50}

(which includes the VX Ct from Bramwell et al. [1963]) is not as credible as the comparable ratio derived from the well-conducted human oral and intra-arterial and intravenous exposure studies of Grob and Harvey (1958) and Sidell and Groff (1974). In addition, the oral exposure studies evaluate the effects of known agent doses (μg/kg). The GB:VX ratio resulting from the oral exposure studies is considered more protective (RP=4.3) than that derived from the direct systemic intra-arterial and intravenous studies (RP =2.7). Of the values derived from available human data, the GB:VX ratio calculated from oral dose exposures needed to achieve RBC-ChE_{50} is the most appropriate for the present application.

With no adjustments for differences in recovery or reversibility (aging), direct application of experimental data from human subjects for the ChE_{50} end point supports a GB:VX RP estimate approximating 4.3. With rounding, the GB:VX RP equals 4.0. Because the ChE_{50} end point is part of the continuum of response for these anticholinesterase compounds, it is consistent to apply the same RP for estimating AEGL-1, AEGL-2, and AEGL-3 values for agent VX.

Until additional data from well-conducted experimental studies are available, the current relative potency approach (RP=4) is reasonable, is supported by existing human experimental data, and meets the requirements of the standing operating procedures for developing AEGLs (NRC 2001).

4.4. Structure-Activity Relationships

Mager (1981) conducted a quantitative structure-activity analysis of organophosphorus compounds having anticholinesterase properties. The toxicity end point used in the analysis was the intraperitoneal LD_{50} value for the mouse. The calculated values were similar to the observed values. The observed values (−log LD_{50}) were 0.70, 0.25,0.22, −0.01, and 2.30 for GB, GD, GA, GF, and VX respectively; the calculated values (−log LD_{50}) were 0.43, 0.19, 0.11, 0.01, and 2.41 for GB, GD, GA, GF, and VX, respectively. In this analysis, agent GB was determined to be 3–4 times more toxic than GD and GA; however, it was noted by Mager (1981) that only the L-enantiomorph of GB was tested and that that isomer is 10–20 times more toxic than the D-isomer. The relative potency of the L-isomer is not necessarily reflective of the relative potency for different mixtures of GB isomers. The optical stability of the isomers can be maintained in the laboratory only under special storage conditions involving solvent solutions and

temperature control (Boter et al. 1966). Those same conditions are not maintained in munition storage or the field.

The toxicokinetics of VX stereoisomers [(±)−] have been examined and preliminary results documented in recent reports from the TNO Prins Maurits Laboratory (Benschop 1999; Benschop et al. 2000). Benschop and his colleagues studied the toxicokinetics of several VX stereoisomers [(±)−] in hairless guinea pigs (intravenous and percutaneous exposures) and marmosets (intravenous exposures only). Following an intravenous dose of 28 μg/kg (marmosets) or 56 μg/kg (guinea pigs), VX was found in the blood at toxicologically relevant levels after 6 h. Detoxification proceeded at a slower rate in marmosets than in guinea pigs. Desethyl-VX was found after incubation of VX in plasma of all species tested; however, because of its slow rate of formation, Benschop et al. (2000) concluded that it would be unlikely that this compound would be present at toxicologically relevant levels after administration of VX in vivo.

4.5. Other Relevant Information

4.5.1. Breathing Rates and Toxicity

For chemicals that are as acutely toxic as the nerve agents, and for which the concentration-response curves are expected to be very steep, a critical factor associated with the estimation of the potential inhalation toxicity is the breathing rate of individuals who might be exposed. In the case of the nerve agents, the vapor concentration producing a similar level of effect can be considerably different depending on the inhalation rate. In studies conducted on 125 human volunteers exposed to nerve agent GB, Oberst et al. (1968) demonstrated that the same end point (50% of RBC-ChE depression) could be attained with 2-min exposures to GB concentrations as high as 16.2–22.7 mg/m^3 (average 20.7 mg/m^3) in men breathing 5.6–8.4 L of air per minute; however, concentrations of only 3.9–4.53 mg GB/m^3 (average 4.19 mg/m^3) were needed to produce the same effect in exercising men breathing 41.5–64.9 L of air per minute. Oberst et al. (1968) reported that the retained dose (mg/kg) in both test groups was very similar.

Minute volumes up to about 25 L/min should cover most situations involving civilian populations; however, breathing rates may be higher under stressful evacuation conditions. Dosimetric adjustments based on

breathing rate are not normally considered by the AEGL protocol (NRC 2001, 57–62). In the case of the nerve agents, such a dosimetric adjustment would not be necessary for the AEGL-1 (and, to some extent, the AEGL-2) values, which are based on a local effects to the eye (miosis) as the most sensitive indicator of direct vapor exposure toxicity (see also Section 2.7 of this document). Changes in breathing rate would not affect this end point.

As is true for AEGL-3 determinations for agent GB, the composite UF applied in the determination of an AEGL-3 for agent VX does not include any adjustment for interspecies differences in dosimetry due to species differences in breathing rates, minute volumes, and body weight. For systemic poisons that are 100% absorbed, the minute volume-body weight normalization method results in a human equivalent concentration approximately 3.5 times greater than that for rats for the same end point (NRC 2001). However, for high exposure levels, such as those at the AEGL-3 level, absorption may be less than 100% and the estimated human equivalent exposure may be excessively high, resulting in an underestimation of the toxicity of the compound (NRC 2001). Another possible dosimetric adjustment is one using the inhaled dose against the body weight raised to the three-fourth power (EPA 1992). This approach is supported by the results of chronic toxicity studies but may not be relevant for acute lethality end points (NRC 2001). When applied to breathing rates, the adjustment predicts that rats would receive a dose about 4 times greater than humans. When this adjustment for breathing rate is combined with the adjustment for toxicity (EPA 1992), the two cancel each other out, and the conclusion is reached that equivalent exposures result in equivalent results in both rats and humans (NRC 2001). Use of the EPA RfC dosimetric method for systemically acting Category 2 gases (gases that are moderately water soluble and intermediate in reactivity and would therefore be distributed throughout the respiratory tract and readily absorbed into the blood stream) results in the prediction that humans would receive a dose ranging from 6,000 to 50,000 times greater than a rodent (depending on the species) for an equivalent exposure (NRC 2001). These numbers are not considered biologically reasonable or scientifically credible by the NRC (2001).

Given the uncertainties surrounding the issue of dosimetric adjustment across species, and the fact that no dosimetric correction would be the most conservative public-health approach, the NAC/AEGL committee decided that it would not use dosimetry corrections across species unless there were sufficient data on a specific chemical to support their use. Dosimetric

adjustments for nerve agents are complicated by the fact that species response to cholinesterase inhibitors are affected to an extent by levels of endogenous enzymes that bind with the inhibitors. Some of these detoxification pathways are present in rodents but not in humans (see Section 4.5.3). Therefore, a dosimetric adjustment alone may be insufficient to account for interspecies differences in response to nerve agents. In consequence, no dosimetric adjustment is required for these compounds, including nerve agents.

4.5.2. Delayed Neuropathy

Exposure to some organophosphate ChE inhibitors results in delayed neurotoxic effects (distal neuropathy, ataxia, and paralysis, which has been referred to as organophosphate-induced delayed neuropathy [OPIDN]) several days to several weeks after exposure. These effects, characterized by axon and myelin degeneration, are not associated with the inhibition of AChE and had been thought to be a consequence of the inhibition (and subsequent aging) of an enzyme known as neuropathy target esterase (NTE) (Abou-Donia 1993; Ehrich and Jortner 2002). As pentavalent phosphorous-containing compounds, the G agents and agent VX may also indirectly generate neurotoxic effects through a noncholinergic mechanism involving the kinase-mediated protein Ca^{2+}/calmodulin kinase II (Ca^{2+}/CaM kinase II) (de Wolff et al. 2002; Abou-Donia and Lapadula 1990). The Ca^{2+}/CaM kinase II protein becomes activated by OP-induced phosphorylation, and reacts (proteoloysis) with the cytoskeletal proteins found in neurofilaments to produce axonal swelling and degeneration in the large-diameter tracts of the spinal cord. The proteolysis and axonal degeneration are accompanied by accumulation of myelin debris, perturbed ionic gradients, and cellular edema (de Wolff et al. 2002).

For some OP compounds, delayed neuropathy can be induced in experimental animals at relatively low exposure levels, whereas for others the effect only is seen following exposure to supralethal doses, when the animal is protected by antidotes from acute cholinergic effects caused by ChE inhibition. In either case, there is evidence that a threshold exists below which delayed neuropathy does not occur. Studies reviewed by Somani et al. (1992) indicate that, in chickens (a species particularly susceptible to delayed neuropathic effects), a 70% decrease in brain NTE activity 24–48 h after exposure is related empirically to the subsequent development of

delayed neuropathy. According to Husain et al. (1995), a minimum of 45% NTE inhibition is associated with delayed neuropathy after multiple exposures.

G Agents

It has been shown that agents GB, GA, and GD inhibit NTE in vitro (Vranken et al. 1982). Supralethal doses of all three G agents produced delayed neuropathy in antidote-protected chickens in vivo (Gordon et al. 1983; Willems et al. 1984). Doses of 120×LD_{50} for agent GA resulted in mild neuropathic signs, and delayed neuropathy was observed at 120–150 ×LD_{50} for GD in a single surviving hen, but not at GD doses of 38×LD_{50}. Delayed neuropathy was also observed in chickens administered agent GB at 30–60×LD_{50}. In all of these challenge tests, nerve agents were administered to adult chickens previously protected from lethality by large antidote doses (Gordon et al. 1983; Willems et al. 1984). Because chickens are considered a sensitive species for this effect, it would appear that the potential for delayed neuropathy would be a concern only for those human individuals surviving a single exposure to concentrations greater than 30× LD_{50} for the G agents. There are also some delayed neuropathy data for animals receiving serial exposures.

Although not comparable to the single, one-time exposure assumption basic to AEGL determinations, the serial exposure data are useful to illustrate the high-concentrations of G agents necessary to induce delayed neuropathy. Signs indicative of delayed neuropathy have been observed in chickens receiving serial subcutaneous injections of one-tenth LD_{50} of agent GB on each of 10 successive days (a total of 1×LD_{50}) (Husain et al. 1995) and in mice exposed to GB vapors at 5 mg/m^3 (one-sixth LD_{50}) for 20 min/d on each of 10 successive days (a total of 1.66×LD_{50}; Husain et al. 1993). Rats receiving daily gavage doses of GB for 90 d at the maximum tolerated (nonlethal) dose (MTD) did not exhibit neuropathy (Bucci et al. 1991; Bucci and Parker 1992). Of the four G agents evaluated in this report, agent GB has the greatest potential for inducing delayed neuropathy after single, large exposures in excess of those necessary to cause death.

Another type of delayed neuropathy that has been associated with exposures to some organophosphate anticholinesterase agents is referred to as an “intermediate syndrome” (Senanayake and Karalliedde 1987; Brown and Brix 1998). Recovery of muscle function after a well-defined cholin

ergic phase has been followed by reappearance of paralysis between 24 and 96 h postexposure (Baker and Sedgwick 1996; Senanayake and Karalliedde 1987). This delayed response has involved respiratory and proximal limb muscles, neck flexors, and motor cranial nerves (Senanayake and Karalliedde 1987). Paralytic symptoms have been documented to persist as long as 18 d, and some cases require ventilatory support (Senanayake and Karalliedde 1987). Intermediate syndrome is considered to be a reversible neuromuscular effect resulting from a nondepolarizing neuromuscular block. For the purposes of AEGL estimation, single fibre electromyographic changes observed in humans following agent GB vapor exposures are considered a subclinical and protective indication of syndrome onset (Baker and Sedgwick 1996).

Agent VX

No clinical or experimental evidence is available to indicate that VX causes delayed neuropathy in humans (see Munro et al. [1994] for review). Delayed neuropathy was not observed in three strains of antidote-protected chickens given a single subcutaneous dose of VX as large as 0.15 mg/kg (estimated to be 5–10 times the lethal level). Repeated intramuscular injections of VX (0.04 mg/kg/d and equivalent to $1.3 \times LD_{50}$ for this species per day, 3 d/wk for 30 d or 5 d/wk for 90 d) also did not produce any signs of OPIDN (Goldman et al. 1988; Wilson et al. 1988). For comparison, the LD_{50} value for an intramuscular injection of VX in chickens is about 0.03 mg/kg (Goldman et al. 1988).

In 90-d subchronic studies conducted on Sprague-Dawley rats, Goldman et al. (1988) found no incidence of tissue degeneration in brain, spinal cord, or peripheral nerves that could be associated with daily subcutaneous injections of VX at up to 4 μg/kg for 5 d/wk. However, in tests conducted on rats, Lenz et al. (1996) found that continuous subcutaneous exposure (via an osmotic pump) to 57 μg/kg/d (1.3 times the subcutaneous LD_{50} of 45 μg/kg) for 14 d resulted in 75–90% reduction in NTE in the brainstem, midbrain, and soleus muscle. Myopathy was seen in the soleus muscle of the test animals.

There is no clinical or experimental evidence that agent VX induces a delayed neuropathy of the “intermediate syndrome” type.

In summary, delayed neuropathy was not observed in three strains of antidote-protected chickens given a single subcutaneous dose of VX equiv

alent to 5–10 times the lethal dose. Further, repeated supralethal intramuscular injections of VX (each injection being equivalent to 1.3 times the LD_{50}) for either 3 d/wk over 30 d or 5 d/wk over 90 d produced no signs of organophosphate-induced delayed neuropathy (Goldman et al. 1988; Wilson et al. 1988). It is true that, in rats, continuous subcutaneous exposure via osmotic pump to a *daily* supralethal dose equivalent to 1.3 times the subcutaneous LD_{50} for 14 d is reported to generate myopathy in the soleus muscle (Lenz et al. 1996). Nevertheless, application of the Lenz et al. (1996) results seems appropriate only for individuals who survive exposures to lethal concentrations (which are well above final AEGL-3 values).

4.5.3. Intra- and Interspecies Variability in Esterase Activity and Response to Nerve Agents

Intraspecies Variability

Differences between individuals in blood cholinesterase activity may affect their susceptibility to the toxic effects of nerve agents. It has been shown that a small subpopulation of men and women possess genetically determined variants in their plasma ChE resulting in very low activity levels (Harris and Whittaker 1962; Lehmann and Liddell 1969) (see also Jokanović and Maksimović [1997] for review). Studies reviewed by Bonderman and Bonderman (1971) indicate that homozygous individuals have plasma-ChE activity reduced to less than 25% of the normal value. For heterozygous individuals, mean plasma-ChE activity is 64% of normal (range 28–114%) (Lehmann and Liddell 1969). Morgan (1989) reported that about 3% of individuals may have genetically determined low levels of plasma cholinesterase and may therefore be unusually sensitive to some anticholinesterase compounds. The frequency of the atypical homozygous phenotype is estimated at 0.025% (Hayes 1982).

Several studies indicate that plasma- and RBC-ChE activity is significantly lower in women than in men (Rider et al. 1957; Reinhold et al. 1953; Augustinsson 1955; Kaufman 1954; all as cited in Hayes 1982 and Wills 1972). Gender differences of 10% in plasma- or RBC-ChE activity have been reported (Wills 1972). Plasma-ChE activity may also be depressed in pregnant women and in individuals with liver disease, heart disease, allergic conditions, and neoplasms (Wills 1972). Such individuals may also be

at a greater risk from exposure to OP compounds. Although some investigators consider gender differences in plasma ChE activity to be confined to young persons (Shanor et al. 1961), data are available suggesting that adult females may be more susceptible to nerve agents than males. Yokoyama et al. (1998c) reported vestibulocerebellar effects (increased postural sway) in a small population of patients tested 6–8 mo after being exposed to agent GB (sarin) during the Tokyo subway terrorist attack. Both female and male patients (nine of each gender) had similar levels of plasma cholinesterase inhibition following the attack, and in both genders, postural sway was correlated with plasma-ChE activity; however, only in females was the increase in sway significantly greater than controls.

Females are here considered to be part of the susceptible subpopulation. In the Mioduszewski et al. (2000, 2001, 2002a,b) studies on rats, females were statistically more sensitive than males for the lethality end point. For agent GF, LCt_{50} values were generally lower in adult female rats than in adult male rats (Anthony et al. 2002). The observed increased susceptibility of females is taken into account by the intraspecies uncertainty factor (UF) for susceptible subpopulations in AEGL estimation. Additional gender comparisons found in the literature are included in Table 1–25.

While the biological role of plasma cholinesterase is at present unknown, it is acknowledged that plasma cholinesterase may likely serve as a buffer to offset the binding of nerve agents (and preferential binding of agent VX) to RBC-AChE. For example, pretreatment with human plasma cholinesterase has protected laboratory rats (Ashani et al. 1993) and monkeys (Raveh et al. 1997) from lethal and other acute toxic effects of VX exposure. Thus, variability in plasma cholinesterase activity is a parameter of concern for characterization of population susceptibility to nerve agent exposure.

As discussed in Section 4.1, A-esterases (paraoxonase/arylesterase) present in the blood and liver are also capable of hydrolyzing phosphate esters (Cashman et al. 1996; Davies et al. 1996; Wang et al. 1998; and Pond et al. 1995). Further, paraoxonase is known to be polymorphic in human populations, and individuals express widely different enzyme levels (see Section 4.1) (LaDu et al. 1986, as cited in Davies et al. 1996; Furlong et al. 1988, 1989; Kujiraoka et al. 2000).

Individuals expressing certain isomeric forms of the enzyme with low hydrolyzing activity are considered to be more susceptible to organophosphate anticholinesterase poisoning (Yamasaki et al. 1997). The polymor

TABLE 1–25 Comparison of Acute (1–10 min) Lethal Inhalation Toxicity Values for G Agents for Male and Female Rats

	Toxicity Value LCt_{50} (mg·min/m^3)								
	Agent GB			Agent GD			Agent GF		
Species	Females	Males	Ratio F:M	Females	Males	Ratio F:M	Females	Males	Ratio F:M
Rat (5-min)[a]	164	230	0.71						
Rat (10-min)[a]	181	226	0.80						
Rat (1-min)[b]	118	220	0.54	135	196	0.69	110	181	0.61
Rat (10-min)[c]	184	231	0.80				253	368	0.69
Geometric Mean			0.70			0.69			0.64

Note: Entries from primary sources and known experimental data.
[a]Mioduszewski et al. (2000, 2001, 2002a).
[b]Callaway and Blackburn (1954).
[c]Mioduszewski et al. (2001) for agent GB; Anthony et al. (2002) for agent GF; 24-h lethality.

phic paraoxonase gene (PON1) has an important role in the detoxifying metabolism of nerve agents and OP insecticides. The $PON1_{R192}$ paraoxonase isoform hydrolyzes agents sarin (GB) and soman (GD) slowly compared to the $PON1_{Q192}$ isoform (Furlong et al. 2002; Davies et al. 1996). The human population can be organized into three PON1*192 genotypes: $PON1_{Q192}$ homozygotes; heterozygotes; and $PON1_{R192}$ homozygotes (Furlong et al. 2002; Allebrandt et al. 2002). Frequency distributions of the PON1*192 variants have been examined in ethnically diverse populations (Allebrandt et al. 2002). The allele expressing low activity for agent GB and agent GD hydrolysis ($PON1_{R192}$) is significantly more frequent in African-Americans (sampled in Brazil and North America) and Asians (sampled in China, Japan, and Canada) than in individuals of Indo-European origin (sampled in East India, Turkey, Canada, Russia, Germany, North America, England, France, the Netherlands, Brazil). Nevertheless, Furlong et al. (2002) point out that "genotyping alone provides no information about PON1 levels, which can vary up to 13-fold between individuals" (homozygous for the low-activity allele) (see also Furlong et al. [1989] and Davies et al. [1996]).

Some investigators have previously considered that low levels of paraoxonase in newborns may contribute to the observed sensitivity of newborn rats to organophosphate insecticides (Benke and Murphy 1975; Burnett and Chambers 1994, as cited in Davies et al. 1996). A recent investigation (Chanda et al. 2002) presents in vitro and in vivo evidence that carboxylesterases "are critical for explaining age-related sensitivity" of rat pups to the OP insecticide chlorpyrifos. Further, the presence of low carboxylesterase activity, although important, does not sufficiently characterize the greater susceptibility of rat pups to neurotoxic effects of certain OP insecticides (Chanda et al. 2002).

Distribution of the low sarin-hydrolysis allele ($PON1_{R192}$) appears to be somewhat ethnically related. The Japanese population has a higher frequency of the low sarin-hydrolysis isoform (allele frequency of 0.66) (Yamasaki et al. 1997) than Caucasian groups documented in the literature (0.24 to 0.31) (Serrato and Marian 1995; Ruiz et al. 1995; Antikainen et al. 1996).

Carboxylesterases are another enzyme group capable of binding with certain OP compounds and are present in human erythrocytes and monocytes as well as in human liver, kidney, lung, skin, and nasal tissue (Cashman et al. 1996; Chanda et al. 2002; Kaliste-Korhonen et al. 1996; Munger et al. 1991). As detailed in Section 4.1, the detoxification potential

of carboxylesterases is multifaceted and is an area requiring further experimental characterization.

Interspecies Variability

Differences exist among animal species in the types of esterases found in the blood as well as in their relative activity, and those differences may affect a species' susceptibility to specific OP compounds. Baseline RBC-AChE activity in humans is slightly higher than that in monkeys but much higher than levels measured in sheep, rats, and other species (see Table 1– 26) (Ellin 1981). Species differences also exist in plasma cholinesterase levels. In humans, about 50% of the total blood ChE consists of plasma ChE (Osweiler et al. 1985). Plasma-ChE activity constitutes about 40% of the total blood ChE in dogs, about 30% in rats, and 20% in monkeys, but only 10% in sheep, horses, and cows (Wills 1972). Cohen et al. (1971) reported that plasma ChE activity in humans was 2 times greater than that in mice and 4 times greater than that in rats. Because of its more rapid turnover time when compared with RBC-AChE, plasma ChE may function as a repository and primary detoxification pathway for many OP compounds. This logic also applies to the carboxylesterases, discussed more fully in the earlier section on intraspecies variability.

It is acknowledged that the CaE profile in humans is not well known and that there are few data from which to characterize the contributions that CaE may make to human protection from anticholinesterase poisoning. Chanda et al. (2002) consider that full characterization of CaE amount, affinity, and IRE in human tissues will be necessary before accurate predictions can be made regarding CaE detoxification potential following anticholinesterase exposures to humans. Interspecies variation in response to some nerve agents may be accounted for largely by carboxylesterase binding (Somani et al. 1992). The G agents readily bind with carboxylesterases (Fonnum and Sterri 1981; Jokanović 1989; Clement 1994; Maxwell et al. 1987; Jokanović et al. 1996), and Maxwell (1992) demonstrated that endogeneous carboxylesterase activity provided rats with protection against the lethal effects of agents GA, GB, and GD, but not VX. In rodents, detoxification of G agents might be accounted for largely by carboxylesterases binding, and in the case of GD, binding appears to occur specifically with the most toxic stereoisomer of the agent (Cashman et al. 1996). Inhibition of carboxylesterase activity significantly increased the

acute toxicity of GD, GB, and GA to laboratory animals (Clement 1984; Jokanović 1989; Maxwell et al. 1987), and induction of carboxylesterase activity by pretreatment with phenobarbital substantially reduced the acute toxicity of GD and GA, but not GB (Clement 1983, 1984; Jokanović 1989). In contrast, selective inhibition of acetylcholinesterase or butyrylcholinesterase had no effect on the acute toxicity of GD to mice (Clement 1984). Because rodents have high levels of plasma carboxylesterases, they may be less susceptible to the G agents than humans.

TABLE 1–26 Baseline RBC-ChE Activity in Different Species[a]

Species	RBC-ChE Activity (μmol/mL/min)	Optimum Substrate Concentration (M)
Human	12.6	2×10–3
Monkey	7.1	2×10–3
Pig	4.7	1×10–3
Goat	4.0	2×10–3
Sheep	2.9	2×10–3
Mouse	2.4	2×10–3
Dog	2.0	2×10–2
Guinea pig	2.7	2×10–3
Rabbit	1.7	5×10–3
Rat	1.7	5×10–3
Cat	1.5	5×10–3

[a]Ellin (1981); Acetylthiocholine iodide concentration for maximum RBC-ChE activity.

Interspecies variability in response to nerve agents can be evaluated in terms of lethal and nonlethal end points.

G-series Agents

Available experimental agent GB LCt_{50} data for the monkey, dog, and rat are presented in Table 1–13. Data for rats (Table 1–25) show that females of these species are more susceptible than males. Comparisons of female rat LCt_{50} values with those of dogs and monkeys indicate that, in

terms of lethality, adult female SD rats are less susceptible to agent GB than adult dogs or monkeys by approximate factors of 2.0 to 4.0. Because rats are a CaE-rich species and dogs and monkeys were once thought to possess no plasma carboxylesterase (Augustinsson 1959), these differences in susceptibility may be due, in part, to species differences in CaE.

In the case of human lethality estimates, Bide et al. (1999) estimated GB inhalation toxicity values for humans by application of allometric model extrapolation from extensive experimental animal data. The calculated 2-min adult human LCt_{50} was approximately 31 mg·min/m^3, equivalent to a 2-min LC_{50} of 15.5 mg/m^3. In contrast, the 2-min LC_{50} for female SD rats is 52 mg/m^3 (derived from a 2-min LCt_{50} of 104 mg/m^3 as reported by Mioduszewski et al. [2000, 2001, 2002a]). Therefore, the ratio of the 2-min LC_{50} values for female rats and humans is approximately 3.4 (52/15.5). This comparison indicates that, when challenged with a lethal concentration of GB vapor, adult female SD rats are likely to be more resistant than adult humans by a factor between 3.0 and 3.5.

Few comparative studies have been conducted for nonlethal end points. However, some information is available on the miotogenic potency of agent GB in several species, including humans. In a study conducted by Johns (1952), 128 adult male volunteers were exposed to agent GB concentrations ranging from 0.05–3.0 mg/m^3 for 2–20 min in an exposure chamber. Regression analysis of 150 observations, including 55 controls, indicated that the point at which a 50% decrease in pupil diameter would be attained was approximately 4.1 mg·min/m^3, with 90% confidence limits of about 2.7 and 5.7 mg·min/m^3. At the lowest test exposure level (0.05 mg/m^3 for 20 min, equal to a Ct of 1 mg·min/m^3) there was a mean maximum decrease in pupil diameter of 0.82 mm in the right eye and 1.00 mm in the left eye (total of eight observations) compared with 0.36 mm in the right eye and 0.33 mm in the left eye in controls (55 observations). Although mild miosis (defined by the author as a decrease of 1 to 2 mm in pupil diameter) was observed in some subjects at a Ct of 1.0 mg·min/m^3, other subjects exposed to the same Ct exhibited mean maximal pupil decreases of <1 mm.

Callaway and Dirnhuber (1971) evaluated the "miotogenic potency" of GB vapor in humans and rabbits exposed to GB "under goggles" (62 miosis responses in 26 human volunteers and 43 miosis responses in 10 albino rabbits). Nevertheless, it is understood that agent measurements collected during this study were hampered by the limited capabilities and techniques for determining agent vapor concentrations in the early 1970s. Further, when compared with current low-light digital methods, the protocols em

ployed to measure rabbit miosis in Callaway and Dirnhuber (1971) are considered semisubjective. In addition, the study documentation does not fully report miosis incidence in the agent-exposed rabbit population. An airstream of GB vapor (flow rate 0.1 L/min) was delivered to the space enclosed by each goggle. The unexposed pupil area of each eye was the baseline for pupil area decrement determinations for each eye. Exposure time periods ranged from 10 min to 5 h. Callaway and Dirnhuber (1971) reported a 50% decrement in pupil area in humans at a Ct of 3.13 mg·min/ m^3 (with 95% confidence limits of 2.15–4.57 mg·min/m^3) and in rabbits at a Ct of 2.33 mg·min/m^3 (with 95% confidence limits of 1.65–3.31 mg·min/m^3). A 90% decrement in pupil area occurred in humans at a Ct of 13.85 mg·min/m^3 (with 95% confidence limits of 6.00–32.02 mg·min/m^3) and in rabbits at a Ct of 7.68 mg·min/m^3 (with 95% confidence limits of 4.90–19.50 mg-min/m^3). Callaway and Dirnhuber (1971) reported that comparison of the values for 90% area decrement suggests that the human eye "may be somewhat less sensitive to GB than the rabbit eye in that it appears to be more difficult to produce a maximal miosis with low concentrations of GB vapor in humans than in rabbits, but this has not been validated statistically."

Van Helden et al. (2001, 2002) exposed marmosets and guinea pigs (whole-body) to GB vapor concentrations at 0.05 to 150 μg/m^3 for 5 h. In guinea pigs, the LOAEL for miosis (5% decrement in pupil size compared with controls; estimated to be equivalent to approximately 10% decrement in pupil area; $p<0.05$) was reported to be 1.8±0.3 mg·min/m^3. In marmosets, the LOAEL for miosis (10% decrease in pupil size compared with controls; estimated at approximately 20% decrement in pupil area; $p< 0.05$) was reported to be 2.5±0.8 mg·min/m^3. Van Helden et al. (2001, 2002) reported that the guinea pig and marmoset LOAEL values were not significantly different.

Mioduszewski et al. (2002b) exposed young adult male and female SD rats (whole-body) to a range of GB vapor concentrations (0.01–0.48 mg/m^3) for three time durations (10, 60, and 240 min). The results (ECt_{50} for miosis) are summarized in Table 1–15.

The results of the Callaway and Dirnhuber (1971), van Helden et al. (2001, 2002) and Mioduszewski et al. (2002b) studies suggest that, in terms of miosis, the response of mammalian eyes appears to be quantitatively very similar across species (including humans).

Agent VX

Interspecies differences in susceptibility to VX have also been reported. In subacute inhalation studies conducted on rats, mice, guinea pigs, and rabbits (exposures were 6 h/d, 5 d/wk, for 2 wk), Crook et al. (1983) determined from calculated LCt_{50} values that mice (LCt_{50}=0.9 mg·min/m^3) were more sensitive to VX than rats (LCt_{50}=24.9 mg·min/m^3), and rats were more sensitive than guinea pigs (LCt_{50}=238.6 mg·min/m^3). Rabbits were more resistant than guinea pigs.

The detoxification potential of endogenous carboxylesterase to protect against the lethal effects of nerve agent exposure was tested by Maxwell (1992) in (male) SD rats. Nerve agents GA, GB, GD, or VX in isotonic saline were administered by subcutaneous injection. The degree of in vivo CaE inhibition was measured in the plasma, lung, and liver of exposed rats. In vivo protection provided by endogenous CaE was estimated by comparing differences in LD_{50} following nerve agent exposures to rats with inhibited CaE activity (following administration of the probe, 2-(O-cresyl)-4H-1,3,2-benzodioxaphosphorin-2-oxide) versus nerve agent exposures to rats without inhibited CaE activity. Maxwell determined that endogenous CaE in the rat provided no significant protection against in vivo lethal exposures to nerve agent VX under the experimental protocol employed; furthermore, Maxwell concluded that "CaE detoxification does not appear to be important" against exposures to lethal concentrations of agent VX.

In conclusion, the SD rat in vivo experimental results of Maxwell (1992) indicate that endogenous CaE in this species confers no protection against lethal exposures of nerve agent VX. Thus, rats exposed to VX should not be considered more robust than other species possessing a different CaE profile (e.g., humans).

4.5.4. Unique Physicochemical Properties

As discussed by Somani et al. (1992), organophosphate nerve agents consist of stereoisomers resulting from the presence of a chiral phosphorus atom in the molecule. Limited data (mainly from studies with agents GD and GB) indicate that the stereoisomers may differ considerably in their toxic potency. In general, most toxicity studies have utilized racemic mixtures of these agents.

The volatility of agent VX is 10.5 mg/m^3 at 25 °C (DA 1990). The Department of the Army considers agent VX to be "about 2,000 times less volatile than [nerve agent] GB" (DA 1990). A volatility of 3.0±0.5 mg/m^3 was reported for a temperature of 25 °C in tests in which the vapor phase was in equilibrium with the aerosol phase (Frostling 1974).

4.5.5. Concurrent Exposure Issues

Two issues might be of concern: (1) simultaneous exposure to multiple nerve agents or related organophosphate compounds, and (2) simultaneous exposure through multiple exposure pathways.

Multiple Exposures to Similar Chemicals

Because of their similarity in mechanism of action, it can be expected that the toxic effects of the nerve agents would be additive. Clement (1994) and Luo and Liang (1997) reported that the toxicity of agents GB and GF were basically additive when administered together by subcutaneous injection to mice. Nevertheless, the various nerve agents are deliberately stored in separate locations and will undergo demilitarization and destruction at separate times. Furthermore, the agents are deliberately stored and secured separately prior to destruction. Thus, the chance for the release of more than one agent while under storage or during the disposal process is minimal.

The acute toxicity for numerous organophosphate insecticides in current use is identical to that of the nerve agents (i.e., initiated by cholinesterase inhibition). The vapor concentrations of insecticides causing acute toxic effects are considerably higher than nerve agent vapor concentrations producing the same end points. Information on lethality levels for some organophosphate insecticides, listed on the Registry of Toxic Effects of Chemical Substances (RTECS) (NIOSH 1999), are shown in Table 1–27. The most acutely toxic of the insecticides listed in Table 1–27 is methyl parathion, for which a 4-h LC_{50} value of 34 mg/m^3 has been reported for rats. In comparison, the 10-min LC_{50} values for rats for agents GA, GB, and GD are 45 mg/m^3, 22 mg/m^3, and 23 mg/m^3, respectively. Using a direct linear extrapolation, the corresponding 4-h LC_{50} values can be estimated to be 1.9 mg/m^3 for GA, 0.92 mg/m^3 for GB, and 0.95 mg/m^3 for GD,

TABLE 1–27 Inhalation Lethality Values for Organophosphate Pesticides

Chemical	Species	Exposure Time (h)	LC_{50} (mg/m^3)	Reference
Tetraethyl dithiopyrophosphate	Rat (f) Rat (m)	4 4	38 59	Kimmerle and Klimmer 1974
Methyl parathion	Rat	4	34	EGESAQ 1980
Parathion	Rat	4	84	AMRL 1977
Phosmet	Rat	4	54	Izmerov et al. 1982
Pirimiphos-methyl	Rat	4	>150	Kagan et al. 1983
Methamidophos	Rat	4	162	Hartley and Kidd 1983–1986
Disulfoton	Rat	NA	200	Klimmer 1971
Ethion	Rat	NA	864	FCH 1991
Naled	Mouse	6	>1,500	Hartley and Kidd 1983–1986
Fonophos	Rat	1	1,900	Hartley and Kidd 1983–1986
Acephate	Mouse	5	>2,200	Berteau and Chiles 1978

Abbreviation: NA, not available.

or approximately 15- to 30-fold more toxic than methyl parathion. The toxicity of VX is considerably greater; the 10-min LCt_{50} value in mice is only 4 mg·min/m^3. Thus, the organophosphate insecticides are considerably less potent than nerve agents.

Comparison of the large differences in LC_{50} values between the G agents, agent VX, and commercial insecticides illustrates that the effects of concurrent exposure would be dominated by the more potent nerve agents. In consequence, concurrent exposure is of far less significance than exposure to each nerve agent alone.

Multiple Exposure Through Different Exposure Pathways

Nerve agents can be absorbed through the skin as well as through the respiratory tract. The extent of skin absorption of a vapor depends on the physicochemical characteristics of the agent and the presence of moisture on the skin. A comparison of the relative toxicity of the nerve agent vapors through inhalation and skin absorption can be made by evaluating the reported LCt values for each pathway.

In studies on human subjects, Freeman et al. (1954) reported that doses up to 400 mg of liquid agent GA applied to the skin of the forearm (5 mg/kg) and allowed to evaporate to dryness caused no clinical signs but resulted in a 30% decrease in RBC-ChE activity. The degree of liquid versus vapor absorption through the skin was not measured in the Freeman et al. (1954) study.

Although the human exposure study of Bramwell et al. (1963) might have provided potential percutaneous ECt_{50} values for severe or threshold effects in humans, the study is flawed by a defective protocol (no reliable estimate of agent exposure to the subjects; see discussion in Section 2.2.2).

Information on the percutaneous toxicity of the G series nerve agents and agent VX was reviewed by a subcommittee of the National Research Council Committee on Toxicology in *Review of Acute Human-Toxicity Estimates for Selected Chemical-Warfare Agents* (NRC 1997). Following evaluation of relevant human and animal studies, the NRC summarized human toxicity estimates. Differences between ECt_{50} values for mild effects resulting from vapor inhalation exposures (GA and GB, 0.5 mg·min/m^3; GD and GF, 0.2 mg·min/m^3) and the ECt_{50} values for threshold effects resulting from percutaneous vapor exposures (GA, 2,000 mg·min/m^3; GB, 1,200 mg·min/m^3; GD and GF, 300 mg·min/m^3) are all in excess of 10^2. The NRC

(1997) considered the GD and GF percutaneous vapor values to be in need of further research and the inhalation vapor estimates to be "low." Nevertheless, the NRC recommendations suggest that, for mild effects, the vapor inhalation pathway is several orders of magnitude (approximately 10^3) more effective than the percutaneous vapor pathway. There are similar order-of-magnitude differences for severe effects (NRC 1997).

In Chapter 6 of NRC (1997), "Review of Acute Human-Toxicity Estimates for VX," relevant human and animal studies are summarized. The NRC reported percutaneous vapor VX LCt_{50}s of 11.5 mg·min/m^3 for mice and 100–150 mg·min/m^3 for clipped goats (body-only) (Koon et al. 1960). In comparison, a whole-body (inhalation and percutaneous) vapor LCt_{50} of 9.2 mg·min/m^3 was reported for goats; the comparable value for mice was 4.0 mg·min/m^3 (Koon et al. 1960). It appears that VX vapor exposures involving inhalation are more effective in causing lethality than percutaneous vapor exposures alone; the difference in effectiveness for the lethality end point is approximately 3 for mice and between 11 and approximately 16 for goats.

Human toxicity estimates listed by NRC (1997) include a VX ECt_{50} value of 0.09 mg·min/m^3 for mild effects resulting from vapor inhalation exposures and an ECt_{50} value of 10 mg·min/m^3 for threshold effects resulting from percutaneous vapor exposures. The latter value was considered by NRC to have an associated low degree of confidence, and further research was recommended. However, these recommendations suggest that for mild effects, the vapor inhalation pathway is several orders of magnitude more effective than the percutaneous vapor pathway. For severe effects, the NRC (1997) presented an ECt_{50} value of VX at 10 mg·min/m^3 for vapor inhalation exposures and an ECt_{50} value of 25 mg·min/m^3 for percutaneous vapor exposures as interim values (low confidence, further research recommended), indicating that for this end point, the inhalation pathway is 2.5 times as effective as the percutaneous pathway for the severe effects ECt_{50}.

The issue of differential toxicity associated with physical states of the same compound has been illustrated in the case of certain industrial compounds; an example is *n*-butyl acetate (OXO Process Panel 1995). *n*-butyl acetate has commercial use in fine furniture manufacture as a vehicle in spray finish application. In studies of rats exposed to *n*-butyl acetate atmospheres generated by either evaporation (vapor exposure) or "atomization" (submicron aerosol exposure), lethality was profoundly different and almost entirely dependent on the physical state of *n*-butyl acetate to which

rats were exposed. Irritation and hypoactivity were noted in animals exposed to *n*-butyl acetate as the vapor (6,800 ppm for 4 ho), but the animals recovered within 1 d and went on to gain weight during the 14-d recovery period. Exposure to comparatively low concentrations (approximately 150 ppm v/v) of *n*-butyl acetate as the aerosol resulted in severe lung damage and mortality. During industrial spray application of finishes in wood furniture manufacture, aerosol particles of *n*-butyl acetate are extremely short-lived, and measurement of worker breathing zone exposure found only the vapor (OXO Process Panel 1995). In consequence, it was determined that toxicity information on the vapor form of *n*-butyl acetate was more appropriate than information on the aerosol form in establishing *n*-butyl acetate occupational exposure limits (ACGIH 1996).

The above examples support the need for research characterizing the emissions profile expected during VX release. Parameters essential to accurate quantification by modern methods and protocols include the following: generation and yield of vapors versus aerosols; rate of aerosol conversion to the vapor; atmospheric degradation half-times; deposition rates; and rates of degradation as influenced by humidity, temperature, and ultraviolet light. Until these parameters are more fully characterized in determinations of differential toxicity of VX vapor and aerosols, AEGL determinations will necessarily be based on the assumption of exposure to VX as the vapor.

It is acknowledged that droplets and/or aerosols may be present during certain release events. Nevertheless, the community emergency preparedness need for guidelines is presently focused on vapor exposure. There is interest and potential for developing a comparable guideline for exposures to nerve agent aerosols at some future time.

4.5.6. Critical Effect End Point

Blood cholinesterase levels are too variable to use as critical effect end points in deriving AEGLs for the nerve agents. Although there are some estimates of enzyme inhibition levels that are associated with acute effects, individual response will vary not only with baseline ChE levels but also with certain characteristics of physiological status (e.g., anemia, liver dysfunction or infection, pregnancy, etc.) which are transient and thus result in dynamic individual susceptibility through time (Lessenger and Reese 1999; Bakerman 1984; Rider et al. 1957; Ciliberto and Marx 1998; Haboubi and Thurnham 1986; Phillips 1995). Local effects on the eyes

(miosis) and upper respiratory tract (rhinorrhea) are more sensitive and consistent indicators of exposure. A number of investigators consider both miosis and rhinorrhea to be early signs of exposure to cholinesterase inhibitors. The presence of rhinorrhea can be indicative of inhalation exposure and/or development of systemic effects, although miosis alone in the absence of other signs or symptoms is a local effect to the pupillary muscles of the eye. In consequence, the presence of miosis is considered an appropriately sensitive indicator of direct vapor exposure and has the added advantage of being readily recognized and quantifiable.

The logic of not using ChE depression as a critical effect is consistent with the science policy of EPA's Office of Pesticide Programs (EPA 2000). According to EPA, there is no predetermined percentage of enzyme activity inhibition that separates adverse from nonadverse effects. The weight-of-evidence analysis advocated by this science policy document for selection of critical effects considers first the "clinical signs and other physiological and behavioral effects in humans and animals," after which "symptoms in humans" are considered, and then changes in blood cholinesterase. The recommended sequence is as follows:

1. Clinical signs and other physiological and behavioral effects in humans and animals.
2. Symptoms in humans.
3. Cental nervous system acetylcholinesterase inhibition.
4. Peripheral nervous system acetylcholinesterase inhibition.
5. Red blood cell acetylcholinesterase inhibition.
6. Plasma cholinesterase inhibition in humans and animals.

Miosis can be observed before significant ChE depression can be measured; setting AEGL values on the basis of miosis (a local effect) will protect against significant ChE activity depression (a systemic effect).

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

G-Series Agents

Candidate human data from which to develop AEGL-1 values for the G agents are available in the studies of Harvey (1952) and Johns (1952),

the study of McKee and Woolcott (1949), and the study of Baker and Sedgwick (1996). In the study described by Harvey (1952) and Johns (1952), several male volunteers who were exposed to GB at 0.05 mg/m^3 for 20 min experienced mild effects including miosis, rhinorrhea, and tightness in the chest (see Tables 1–7 and 1–8). Miosis and rhinorrhea were clinically observed. Harvey (1952) and Johns (1952) quantified miosis as the maximal decrease in pupil diameter measured with a modified fixed focus prism telescope in a clinical setting. In the study of McKee and Woolcott (1949), five male subjects were exposed to GB at 0.062 mg/m^3 for 20 min/d without any signs of clinical effects until day 4, when miosis was observed. A single exposure to GB at 0.6 mg/m^3 for 1 min or 0.06 mg/m^3 for 40 min resulted in miosis and slight tightness of the chest. In the Baker and Sedgwick (1996) study, eight healthy male servicemen who were exposed to GB at 0.5 mg/m^3 for 30 min developed miosis and several also exhibited photophobia and dyspnea. In addition, RBC-ChE activity was inhibited to approximately 60% of individual baseline at 3 h and 3 d postexposure, and small but measurable changes occurred in single fibre electromyography (SFEMG) of the forearm. The latter, which were detectable in the lab between 4 and 15 mo postexposure, were not considered clinically significant by Baker and Sedgwick. The SFEMG changes were not detectable after 15–30 mo.

In tests on 125 volunteers, Oberst et al. (1968) observed no signs or symptoms of toxicity in resting men (breathing rate about 7 L/min) following 2-min exposures to an average GB concentration of 20.7 mg/m^3 or in exercising men (breathing rate 50 L/min) following 2-min exposures to an average GB concentration of 4.19 mg/m^3. Linear extrapolation of the lower concentration results in a 30-min exposure to GB at 0.27 mg/m^3, less than the exposure used in the Baker and Sedgwick (1996) study.

The above studies do not agree in identifying the concentration of GB at which effects first appear, and there are even inconsistencies within some of the studies. One possibility for the conflicting results is human variability, since few subjects were used in each study. Another possibility is that the analytical measurements were not accurate. There are no details on analytical procedures in the study of Harvey (1952) or Johns (1952), where it is stated that "known concentrations" were used. The Baker and Sedgwick (1996) study provides a description of the analytical method and indicates that exposure concentrations were verified before and after exposure.

Agent VX

Clearly defined human concentration-response data for low-level inhalation exposures to agent VX are not available. The human toxicity studies that have been conducted with VX are not considered adequate for deriving exposure limits. The study conducted by Bramwell et al. (1963) suggests that an inhalation dose of about 8 µg/kg causes a 50% ChE depression as well as signs of toxicity including miosis, rhinorrhea, and nausea; however, this suspect study involved multiple exposures to the same individuals, short exposure durations (maximum of 7 min), and an experimental protocol (open tunnel rather than exposure chamber) in which the individual exposures to VX may have varied. The Bramwell et al. (1963) study is therefore not considered credible because of its seriously flawed exposure protocol.

Other experimental data indicate that inhalation exposures equivalent to internal doses in the range of 0.01–0.13 µg/kg result in mild signs of toxicity and no change in ChE (Koon et al. 1959). As extrapolated from historical animal data, the human ECt_{50} for miosis has been estimated at 0.09 mg·min/m^3 (Reutter and Wade 1994).

5.2. Summary of Animal Data Relevant to AEGL-1

G-Series Agents

Acute inhalation toxicity data are available for agents GA and GB for several animal species. In most cases, the studies were designed to estimate LCt_{50} values, and they are not directly suitable for application to an AEGL-1 estimation. Several studies, however, have identified minimal effect levels. Van Helden et al. (2001, 2002) reported LOAELs for miosis of 2.5 ±0.8 mg·min/m^3 for marmosets and 1.8 ±0.3 mg·min/m^3 for guinea pigs exposed to agent GB for 5 h. The LOAEL values for miosis in the two species were not statistically different (van Helden et al. 2001, 2002). Mioduszewski et al. (2002b) reported ECt_{50} values for miosis in male and female SD rats exposed (whole-body) to GB vapor for time durations of 10, 60, and 240 min. Miosis was defined by the authors as "post-exposure pupil diameter 50% or less of the pre-exposure pupil diameter." The ECt_{50} determinations for both genders are summarized in Table 1–21.

In studies conducted by Harris et al. (1953), dogs were able to tolerate daily exposures to GB at an average Ct of 10.5 mg·min/m^3 (equivalent to an average concentration of 0.53 mg/m^3 for 20 min), 5 d/wk, for 2 mo. The only reported clinical sign was miosis, which appeared with each exposure but disappeared before the next exposure. However, when each daily exposure was increased to 15 mg·min/m^3, toxic signs (body tremors, dyspnea, loss of muscle control, convulsions) occurred within 7–10 d and several dogs died. Henderson et al. (2000, 2001, 2002), Conn et al. (2002), and Kalra et al. (2002) exposed male F344 rats to GB at 0.2 mg/m^3 or 0.4 mg/m^3 (nose-only) for 1 h/d for 1 d, 5 d, or 10 d, with sacrifices at 1 d after exposure and at 1 mo after exposure. Henderson et al., Conn et al., and Kalra et al. reported that there were no overt signs or symptoms of neurotoxicity (tremors) under non-heat stress conditions at either GB exposure concentration and that single GB exposures "did not alter body weight, breathing patterns, routine brain histopathology, or apoptosis in brain cells."

Agent VX

There are no single exposure studies available for deriving AEGL-1 values for VX. In a nonverifiable study, Crook et al. (1983) reported no signs of toxicity except miosis in rats, mice, guinea pigs, or rabbits exposed to VX vapor concentrations up to 0.0002 mg/m^3 for 6 h/d, 5 d/wk, for 2 wk. A test concentration of 0.004 mg/m^3 resulted in rat and mice mortality. The available animal data indicate that VX does not cause reproductive or developmental toxicity, and there is no evidence suggesting that VX is genotoxic or carcinogenic.

In an examination of miotogenic potency, Callaway and Dirnhuber (1971) consider that agent VX is an order of magnitude more effective than agents GB or GD at producing miosis in the eyes of male and female albino rabbits.

5.3. Derivation of AEGL-1 for Agent GB

The estimation of interim AEGL-1 values relied on the Harvey (1952) study (66 Fed. Reg. 21940 [2001]). Of 14 individuals exposed to the lowest concentration for the longest exposure time (0.05 mg/m^3 for 20 min), the

following signs and symptoms were reported: two headaches, two eye pain, three rhinorrhea, one tightness in the chest, one cramps, one nausea, and two malaise. Of human studies available, this analysis gave the lowest LOAEL of 0.05 mg/m^3 for a 20-min exposure and was chosen as the basis for deriving the interim AEGL-1 values. The miosis effects data of Johns (1952) were considered as supportive. The subjects were male "normal human volunteer" service personnel between the ages of 22 and 59 and under clinical supervision during the periods of exposure as well as for postexposure periods of several months. Derivation of the interim values is detailed in Appendix A.

The final analysis relies on the Mioduszewski et al. (2002b) study of miosis induction to young adult SD rats as the basis for AEGL-1 estimation, with retention of van Helden et al. (2001, 2002; marmosets), Harvey (1952) (humans) and Johns (1952) (humans) as secondary and supportive studies.

The selection of miosis induction as the basis for deriving final AEGL-1 values is supported by the evaluation of a U.S. Surgeon General's review panel on agent exposure limits convened by the Chemical Demilitarization Branch of the National Center for Environmental Health of the Centers for Disease Control and Prevention (CDC) (67 Fed. Reg. 894 [2002]; DHHS 2002). Although the CDC has not yet finalized its position, the review panel generally concluded that cholinesterase activity depression is too variable for application as a critical effect in the estimation of nerve agent exposure limits and that miosis is an appropriate and readily quantified critical effect.

The AEGL-1 values for agent GB were derived from a well-conducted study on adult female Sprague-Dawley rats exposed whole-body in a dynamic airflow chamber to a range of GB vapor concentrations (0.01–0.48 mg/m^3) over three time durations (10 min, 60 min, or 240 min) (total of 283 agent-exposed rats, of which 142 were female and 141 were male) (Mioduszewski et al. 2002b). With the inclusion of range-finding experiments and controls (N=130), a total of 423 rats were employed in this well-conducted study documenting highly credible protocols for GB vapor generation and measurement. A sufficient number of individual animals were exposed at each interval (10 min, 52 female SD rats; 60 min, 35 female SD rats; 240 min, 55 female SD rats). Analysis of rat pupil diameters assessed pre- and postexposure allowed generation of EC_{50} determinations for miosis (defined as a postexposure pupil diameter of 50% or less of the preexposure diameter in 50% of the exposed population). Blood samples

collected from tail vein and heart at 60 min and 7 d postexposure indicated no change from preexposure baseline in monitored blood RBC-ChE, butyrylcholinesterase (BuChE) or carboxylesterase. No other clinical signs were evident throughout the duration of the study. Gender differences (females more susceptible) were statistically significant at 10 min (p= 0.014) and 240 min (p=0.023) but not at 60 min (p=0.054). As the female rat appears to be more susceptible than the male for at least two of the AEGL exposure durations of interest, the AEGL-1 estimations are calculated from the female data set. This data set selection for the most susceptible gender will provide a more protective estimation of AEGL-1. This is a well-defined animal end point in a susceptible gender and is transient, reversible, and nondisabling. (Further details of this study are provided in Section 3.2.3.)

Data from the GB vapor study of nonhuman primates (marmosets; 5 h exposures to GB vapor concentrations of 0.05 to 150 $\mu g/m^3$) (van Helden et al. 2001, 2002) and human volunteers (minimal and reversible effects of miosis, rhinorrhea, headache, etc., after a 20-min exposure to a GB vapor concentration of 0.05 mg/m^3) (Harvey 1952; Johns 1952) are considered secondary and supportive. The human data of Harvey (1952) and Johns (1952) indicate that some adult humans exposed to concentrations within the exposure range tested by Mioduszewski et al. (2002b) would experience some discomfort (headache, eye pain, nausea, etc.) in addition to miosis corresponding to ≤50% pupil area decrement, but no disability (see definition of AEGL-1 provided in NRC [2001]). The studies of Harvey (1952) and Johns (1952) also show that miosis is transient and reversible, with reversibility occurring within hours to days (depending on degree of miosis). This is consistent with other human data documenting miosis after nerve agent vapor exposures. In consequence, with the knowledge that the EC_{50} exhibited by rats in the study of Mioduszewski et al. (2002b) is also transient and reversible, the determination is made that EC_{50} for miosis in female SD rats is an appropriate end point for estimating AEGL-1 values (Mioduszewski et al. 2002b).

Because exposure-response data were unavailable for all of the AEGL-specific exposure durations, temporal extrapolation was used in the development of AEGL values for the AEGL-specific time periods. The concentration-exposure time relationship for many systemically acting vapors and gases may be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5. The temporal extrapolation used here is based on a log-log linear regression of the LC_{01} lethality of GB to female Sprague-

Dawley rats (Mioduszewski et al. 2000, 2001, 2002a) and a log-log linear regression of female SD rat miosis data following GB vapor exposure for time durations of 10 min to 240 min (Mioduszewski et al. 2002b). Regression analysis of the LC_{01} values yields an n value of 1.93 with an r^2 of 0.9948, while regression analysis of the miosis data yields an n value of 2.00 with an r^2 of 0.4335 (24 data points; see Appendix B). Given that all mammalian toxicity end points observed in the data set for all nerve agents represent different points on the response continuum for anticholinesterase exposure, and that the mechanism of acute mammalian toxicity (cholinesterase inhibition) is the same for all nerve agents, the experimentally derived n=2 from the rat lethality and miosis data sets is used as the scaling function for all the AEGL derivations rather than a default value. An n of 1.16 (r^2=0.6704) was calculated for comparison using other data (human volunteer) and other end points (e.g., GB-induced miosis in humans; see Appendix B). However, due to uncertainties associated with some of the exposure measurements in these earlier studies, the Mioduszewki et al. rat data were determined to be the best source of an estimate for n.

Derivation of AEGL-1 Values Using Animal Data

AEGL-1 values can be derived from the data set presented by van Helden et al. (2001, 2002) for GB-induced miosis in marmosets exposed to agent GB vapor for 5 h, as well as the data set presented by Mioduszewski et al. (2002b) for rats exposed to GB vapor for 10, 60, and 240 min.

Van Helden et al. (2001, 2002) reported a LOAEL for threshold miosis of 2.5 ±0.8 mg·min/m^3, and considered miosis to be significantly different (p<0.05) from controls when a 10% decrease in marmoset pupil size was observed (estimated to be equivalent to an approximate 20% decrement in pupil area). Van Helden et al. (2001, 2002) also reported that there was no significant difference between the LOAEL for miosis in marmosets and in guinea pigs. The EPA IRIS database and the NLM Hazardous Substances Databank were searched for additional information on the miotogenic response of marmosets to cholinesterase inhibitors, but no relevant data were found.

The recent miosis and lethality data of Mioduszewski et al. (2000, 2001, 2002a,b) in rats have been subjected to regression analysis (see Appendix B). In consequence, the nonlethality (miosis) and lethality data of Mioduszewski and his colleagues are determined to be the best source of

an estimate for the *n* value for GB response. The Mioduszewki et al. (2000, 2001, 2002a,b) data sets are robust and compound-specific for the most completely characterized G-series nerve agent, agent GB. As outlined earlier, the mechanism of mammalian toxicity for nerve agents is known, and all end points observed in human and animal studies represent a response continuum to anticholinesterase exposure. Accordingly, it is valid to apply an *n* value derived from compound-specific miosis and lethality data to time scaling for nonlethal as well as lethal effects. This position is consistent with that of the recently published science policy of the EPA Office of Pesticide Programs (EPA 2000). Furthermore, this approach is preferable to the use of default values.

For AEGL-1 derivation, an interspecies uncertainty factor (UF) of 1 and an intraspecies UF of 10 were used, resulting in a composite UF of 10. To estimate an interspecies UF, miosis data for a number of species were compared. Van Helden et al. (2001, 2002) exposed marmosets and guinea pigs (whole-body) to GB vapor to estimate a LOAEL for miosis in both species. They determined that there was no significant difference between guinea pigs and marmosets at the 5% level. Contact with leading investigators in the field (H.van Helden, Pulmonary and CNS Pharmacology Lab, TNO, the Netherlands, personal communication; S.Tattersall, Biomedical Sciences Division at Porton Down, United Kingdom, personal communication) was performed to determine availability of experimental data characterizing miosis following nerve agent vapor exposure to mammals. Dr. Tattersall pointed out that Porton Down has not performed systematic measurements of miosis in recent years, and that the only other extant report of relevant data was Callaway and Dirnhuber (1971), cited in this document. These investigators have independently concluded that the miotogenic response of mammalian eyes to agent GB vapor exposure is quantitatively similar across species, including standard laboratory animals (rabbits and guinea pigs), nonhuman primates (marmosets), and humans (please see Table 1–21). In consequence, the interspecies UF for the AEGL-1 end point of miosis in young adult female SD rats is set equal to 1.

The intraspecies UF of 10 used in the derivation of the AEGL-1 is based on the known polymorphic variation in human cholinesterase and carboxylesterase activity that may make some individuals susceptible to the effects of cholinesterase inhibitors such as nerve agents. A factor of 10 was applied for protection of susceptible populations.

The database for agent GB is reasonably complete. Strong arguments for not incorporating an additional modifying factor include the following:

- Data are available for multiple species.
- Data characterizing both lethal and nonlethal end points have been used in the analysis; the end points possess exposure-response data.
- The mechanism of toxicity is known.
- The *n* value is derived from experimental data and is not the default.
- There are no uncertainties regarding reproductive and developmental effects or issues of carcinogenicity.

In consequence, no modifying factor was used in the estimation of AEGL-1 values.

For comparison, from the marmoset data of van Helden et al. (2001, 2002), *k* was derived using a composite UF of 10.

$([0.0083\ mg/m^3]/10)^2 \times (5\ h) = k;$
$k = 3.4 \times 10^{-6}\ mg/m^3 \times h.$

From the experimental data of Mioduszewski et al. (2002b), *k* was derived as follows for the 10-min to 30-min extrapolation:

$([0.068\ mg/m^3]/10)^2 \times (10/60)\ h = k;$
$k = 7.7 \times 10^{-6}\ mg/m^3 \times h.$

For the 4-h to 8-h extrapolation, *k* was derived as

$([0.012\ mg/m^3]/10)^2 \times 4\ h = k;$
$k = 5.8 \times 10^{-6}\ mg/m^3 \times h.$

The Interim AEGL-1 estimates and the estimates from the van Helden et al. (2001, 2002) (marmoset; 5-h exposure) and Mioduszewski et al. (2002b) (female SD rat; 10-min, 60-min, and 240-min exposures) data sets are summarized for comparison in Table 1–28 below. The interim values (66 Fed. Reg. 21940 [2001]) are bolded.

Comparison of AEGL estimates from this rich database for GB vapor-induced miosis in the eyes of mammals exhibits remarkable concordance and corroboration across species. There is little to no change between the interim estimates derived from historical human data (Harvey 1952; Johns 1952; 66 Fed. Reg. 21940 [2001]) and that derived from the female rat miosis data published in 2002 (Mioduszewski et al. 2002b). Any differences are usually a single digit in the fourth decimal place. Estimates based

on marmoset data (a single exposure period of 5 h) differ from the interim values by an approximate factor of 1.5. Given that variation of this magnitude in the AEGL estimates does not reflect response differentiation with any precision, the GB interim values for AEGL-1 are considered adequately representative and protective against miosis resulting from GB vapor exposure to the public. The female rat miosis experiment of Mioduszewski et al. (2002b) is the critical study for final AEGL-1 determination.

TABLE 1–28 Alternate AEGL-1 Estimates for Nerve Agent GB

Time Period	Interim Value (66 Fed. Reg. 21940 [2001])[a] (mg/m^3)	Alternate 1[b] (mg/m^3)	Alternate 2[c] (mg/m^3)
10 min	0.0069	0.0045	0.0068
30 min	0.0040	0.0026	0.0039
1 h	0.0028	0.0019	0.0020
4 h	0.0014	0.00092	0.0012
8 h	0.0010	0.00065	0.0010

Note: n=2; interspecies UF=1 (research staff of TNO and Porton Down consider miosis response in all mammal eyes exposed to nerve agent vapors to be similar across species); intraspecies UF=10 (adjustment for possible susceptible individuals); total UF=10.

[a]Determined using human data from Harvey (1952) and Johns (1952) (20-min exposure). See Appendix A for details of derivation.

[b]Determined using marmoset miosis data from van Helden et al. (2002) (5-h exposures).

[c]Determined using female SD rat miosis data from Mioduszewski et al. (2002b) (10-min, 60-min, and 240-min exposures).

The recommended AEGL-1 values are summarized in Table 1–29. The calculations of exposure concentrations for female SD rats and humans scaled to AEGL-1 time points are shown in Appendix A.

5.4. Derivation of AEGL-1 Values for Agents GA, GD, and GF

The relative potency approach was used to estimate AEGL-1 values for agents GA, GD, and GF. A discussion of the relative toxic potencies for these agents is given in Section 4.3. It was determined that for the end point of miosis, the effect usually observed at the lowest exposure concen

trations, the potency of GA is identical to that of GB. Agents GD and GF are each considered approximately twice as potent as agents GB or GA for miosis, and equipotent to each other for AEGL-1 effects. Thus, the AEGL-1 concentration values for agents GD and GF are equal to 0.5 times the values derived for agents GA and GB (Table 1–30).

TABLE 1–29 AEGL-1 Values for Agent GB (mg/m3 [ppm])

10 min	30 min	1 h	4 h	8 h
0.0069 mg/m^3 (0.0012 ppm)	0.0040 mg/m^3 (0.00068 ppm)	0.0028 mg/m^3 (0.00048 ppm)	0.0014 mg/m^3 (0.00024 ppm)	0.0010 mg/m^3 (0.00017 ppm)

5.5. Derivation of AEGL-1 for Agent VX

Because of inadequacies in the human and animal toxicologic database for agent VX, the present analysis recommends that the AEGL-1 for agent VX be derived from the critical study (Mioduszewski et al. 2002b) for the agent-GB AEGL-1 using a relative potency approach. The experimental protocol for the Mioduszewski et al. (2002b) study is described fully in Section 3.2.1.

A relative potency (RP) of 4 is used to derive the AEGLs for VX. The well-conducted (and clinically supervised) human exposure studies of Grob and Harvey (1958) and Sidell and Groff (1974) report RBC-ChE$_{50}$ values following single oral or intra-arterial/intravenous exposures to GB and VX (see analysis presented in Tables 1–23 and 1–24). Of the values derived from available human data, the GB:VX ratio (RP=4.3, rounded to 4.0) calculated from oral dose exposures needed to achieve RBC-ChE$_{50}$ is the most appropriate for the present application. Details of this logic are provided in Section 4.3. The comparative miosis study of Callaway and Dirnhuber (1971) is considered secondary and supportive of the concept that agent VX is more potent than GB for the miosis end point.

By applying an RP factor of 4 to the miosis data set of Mioduszewski et al. (2002b), the comparative concentrations for VX were estimated to be one-fourth that of GB, or 0.017 mg/m^3, for a 10-min exposure, 0.005 mg/m^3 for a 60-min exposure, and 0.003 mg/m^3 for a 240-min exposure. The VX concentrations were further adjusted by a composite UF of 30; 1 for

interspecies uncertainty (miosis response is similar across species), 10 for intraspecies variability to accommodate known human variation in ChE and carboxylesterase activity (protection of susceptible populations), and a modifying factor of 3 for the sparse VX data set. To derive AEGL-1 values for different time periods (10 min to 30 min and 4 h to 8 h), the data were scaled using the relationship $C^n \times t = k$ (ten Berge et al. 1986). An n value has not been determined experimentally for VX; however, because the primary mechanism of action (cholinesterase inhibition) is the same as that for agent GB, the n value of 2 used in the derivation of the AEGL values for GB is also appropriate for deriving all AEGL values for VX. In consequence, the experimentally derived n=2 from the Mioduszewski et al. (2000, 2001, 2002a,b) rat miosis and lethality data sets for agent GB is here used as the scaling function for the agent-VX AEGL-1 values, rather than a default value. Until additional data from well-conducted experimental studies are available, the current value of n is reasonable, is supported by existing data, and meets requirements of the standing operating procedures for estimating AEGL values (NRC 2001).

TABLE 1–30 AEGL-1 Values for Agents GA, GD, and GF (mg/m3 [ppm])

Agent	10 min	30 min	1 h	4 h	8 h
GA	0.0069 mg/m^3 (0.0010 ppm)	0.0040 mg/m^3 (0.00060 ppm)	0.0028 mg/m^3 (0.00042 ppm)	0.0014 mg/m^3 (0.00021 ppm)	0.0010 mg/m^3 (0.00015 ppm)
GD	0.0035 mg/m^3 (0.00046 ppm)	0.0020 mg/m^3 (0.00026 ppm)	0.0014 mg/m^3 (0.00018 ppm)	0.00070 mg/m^3 (0.000091 ppm)	0.00050 mg/m^3 (0.000065 ppm)
GF	0.0035 mg/m^3 (0.00049 ppm)	0.0020 mg/m^3 (0.00028 ppm)	0.0014 mg/m^3 (0.00020 ppm)	0.00070 mg/m^3 (0.00010 ppm)	0.00050 mg/m^3 (0.000070 ppm)

The 10-min to 30-min extrapolation was

$C^2 \times t = k$;
([0.017 mg/m^3]/30])2×(10/60) h=k;
k=5.0×10^{-8} mg/m^3×h.

The 4-h to 8-h extrapolation was

$C^2 \times t = k$;
$([0.003\ mg/m^3]/30)^2 \times 4\ h = k$;
$k = 4.0 \times 10^{-8}\ mg/m^3 \times h$.

The resulting AEGL-1 values for VX are summarized in Table 1–31. The calculations of exposure concentrations for humans scaled for all AEGL-1 time points are shown in Appendix A.

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

Human data to derive an AEGL-2 for the G agents are provided in the studies of Harvey (1952), Johns (1952), and Baker and Sedgwick (1996). In the Harvey (1952) study an array of signs and symptoms, including headache, eye pain, dimness of vision, twitching of eyelids, rhinorrhea, salivation, throat irritation, tightness in the chest, cramps, nausea, vomiting, giddiness, difficulty in concentrating, and malaise were reported in individuals exposed to GB at 0.3 mg/m^3 for 20 min. Twelve subjects were exposed at this GB concentration—all experienced rhinorrhea, eight suffered from headaches, and seven reported dimness of vision. In the Baker and Sedgwick (1996) study, eight healthy male servicemen who were exposed to GB at 0.5 mg/m^3 for 30 min developed miosis, and several also exhibited photophobia and dyspnea. In addition, RBC-ChE activity was inhibited to approximately 60% of individual baseline at 3 h and 3 d postexposure, and small but measurable changes occurred in single fibre electromyography (SFEMG) of the forearm. The latter effects, which were detectable in the lab between 4 and 15 mo postexposure, were "not significantly different from the control value," with "control" defined as preexposure baseline readings for each individual subject (Baker and Sedgwick 1996). The SFEMG changes were not detectable after 15–30 mo.

6.2. Summary of Animal Data Relevant to AEGL-2

Animal inhalation data are insufficient to derive AEGL-2 values.

TABLE 1–31 AEGL-1 Valuesa for Agent VX (mg/m3 [ppm])

10 min	30 min	1 h	4 h	8 h
0.00057 mg/m^3 (0.000052 ppm)	0.00033 mg/m^3 (0.000030 ppm)	0.00017 mg/m^3 (0.000016 ppm)	0.00010 mg/m^3 (0.0000091 ppm)	0.000071 mg/m^3 (0.0000065 ppm)

[a]The AEGL values are for vapor exposures only.

6.3. Derivation of AEGL-2 for Agent GB

The present analysis applies the Baker and Sedgwick (1996) study as the basis of the AEGL-2 values. Of the human studies conducted on GB that were available for evaluation, the Baker and Sedgwick study is recent, was conducted following a rigorous experimental protocol, and used modern analytical methods for determining the exposure concentrations (GB at 0.5 mg/m^3 for 30 min). Furthermore, this study was performed under Helsinki accords and clinical supervision and was conducted with the cooperation of fully informed human subjects (*N*=8, "fit male servicemen"). The observed effects included miosis in eight of eight subjects, dyspnea and photophobia in some individuals (number not given), inhibition of RBC-ChE to approximately 60% of individual baseline at 3 h and 3 d postexposure in (eight of eight subjects), and small but measurable changes in single fibre electromyography (SFEMG) of the forearm (in five of eight subjects). Nevertheless, the fact that the SFEMG abnormalities were detectable in the lab between 4 and 15 mo postexposure makes these effects long-lasting, and they are therefore included under the definition of AEGL-2.

Respiratory effects resolved within minutes, and visual effects resolved within 48 h. The SFEMG changes noted in the study were not clinically significant, and were not detectable after 15–30 mo. Baker and Sedgwick considered SFEMG changes to be a possible early indicator or precursor of the nondepolarising neuromuscular block found associated with intermediate syndrome paralysis in severe organophosphorous insecticide poisoning cases (Senanayake and Karalliedde 1987). The study concluded that these electromyographic changes were persistent (>15 mo), but that they were reversible and subclinical. Subclinical and reversible effects are not normally included within the definition of AEGL-2 effects. However, because SFEMG changes may be a precursor of intermediate syndrome (see Section

4.5.2), and because of the steepness of the dose-response curve for nerve agents, the use of this end point for establishing AEGL-2 values is considered a protective approach. This concept of added precaution for steep dose-response is consistent with emergency planning guidance for nerve agents previously developed by the National Center for Environmental Health of the Centers for Disease Control and Prevention (Thacker 1994).

As previously described in the development of AEGL-1 values for the G agents (Sections 5.3 and 5.4), an n value of 2 derived from a linear regression of both miosis and lethality data for GB vapor exposure to female SD rats (Mioduszewski et al. 2000, 2001, 2002a,b) is appropriate for use as a scaling function for all nerve agents. AEGL-2 values for exposure times different from the experimental time of 30 min were thus scaled using an n of 2.

A composite UF of 10 was used in the calculation. To accommodate known variation in human cholinesterase and carboxylesterase activity that may make some individuals susceptible to the effects of cholinesterase inhibitors such as nerve agents, a factor of 10 was applied for intraspecies variability (protection of susceptible populations). Because human data were used, an interspecies UF was not required. The database for agent GB is reasonably complete. As was true for the AEGL-1 estimations, there are strong arguments for not incorporating an additional modifying factor. In consequence, no modifying factor was used in the estimation of AEGL-2 values.

From the experimental data, k was derived as

$$([0.5\ \text{mg/m}^3]/10)^2 \times (0.5\ \text{h}) = k;$$
$$k = 0.0013\ \text{mg/m}^3 \times \text{h}.$$

The resulting estimates of AEGL-2 are summarized in Table 1–32.

6.4. Derivation of AEGL-2 Values for Agents GA, GD, and GF

The relative potency approach is used to estimate AEGL-2 values for agents GA, GD, and GF. A discussion of the relative toxic potencies for these agents is given in Section 4.3. It was determined that for the end point of miosis, the effect usually observed at the lowest exposure concentrations, the potency of GA is identical to that of GB. Agents GD and GF are each considered approximately twice as potent as agents GB or GA for

miosis, and equipotent to each other for AEGL-2 effects. Thus, the AEGL-2 concentration values for agents GD and GF are equal to 0.5 times those values derived for agents GA and GB (Table 1–33).

TABLE 1–32 AEGL-2 Values for Agent GB (mg/m3 [ppm])

10 min	30 min	1 h	4 h	8 h
0.087 mg/m^3	0.050 mg/m^3	0.035 mg/m^3	0.017 mg/m^3	0.013 mg/m^3
(0.015 ppm)	(0.0085 ppm)	(0.0060 ppm)	(0.0029 ppm)	(0.0022 ppm)

6.5. Derivation of AEGL-2 Values for Agent VX

Acute inhalation toxicity studies on animals have identified median lethal concentrations; however, these studies are inadequate for deriving AEGL-2 values because of the lack of dose-response data for the appropriate time periods. Some information for agent VX is available from a repeat exposure study in which a VX concentration of 0.004 mg/m^3 for 6 h/d, 5 d/wk, for 2 wk resulted in severe signs of toxicity (tremors, convulsions, salivation, and bloody tears) and 100% mortality of mice, 35% mortality in rats, and 3% mortality in guinea pigs (Crook et al. 1983). Exposure to 0.0002 mg/m^3 under the same experimental protocol resulted in no toxic signs but miosis and ChE depression. The Crook data set is considered nonverifiable. An AEGL-2 effect for a single 6-h exposure would most likely fall within the range of 0.0002 and 0.004 mg/m^3.

There are no definitive data identifying the minimal exposure level at which severe, irreversible, or escape-impairing effects of acute exposure to agent VX would occur. Because of the inadequacy of the human and animal toxicologic database for agent VX, the AEGL-2 for agent VX is derived from the AEGL-2 for agent GB using a relative potency approach.

The Baker and Sedgwick (1996) study of GB vapor exposure in human volunteers is used as the basis of the AEGL-2 values for agent VX, as described in Section 6.3.

By applying a relative potency of 4, the comparable VX exposure is one-fourth that of GB, or 0.125 mg/m^3, for a 30-min exposure. The VX concentration was adjusted by a composite UF of 30; 1 for interspecies uncertainty (human data), 10 for intraspecies variability to accommodate known human variation in ChE activity (protection of susceptible populations), and a modifying factor of 3 for the sparse VX data set. To derive AEGL-2 values for different time periods, the data were scaled using the

relationship $C^n \times t = k$ (ten Berge et al. 1986). An n value has not been determined experimentally for VX. However, because the mechanism of action (cholinesterase inhibition) is the same as that for agent GB, the n value of 2, as used in the derivation of the AEGL values for GB, is also appropriate for deriving AEGL values for VX. In consequence, the experimentally derived n=2 from the Mioduszewski et al. (2000, 2001, 2002a,b) rat lethality data set for agent GB is here used as the scaling function for the agent VX AEGL-2 values, rather than a default value; therefore

TABLE 1–33 AEGL-2 Values for Agents GA, GD, and GF (mg/m3 [ppm])

Agent	10-min	30-min	1-h	4-h	8-h
GA	0.087 mg/m^3 (0.013 ppm)	0.050 mg/m^3 (0.0075 ppm)	0.035 mg/m^3 (0.0053 ppm)	0.017 mg/m^3 (0.0026 ppm)	0.013 mg/m^3 (0.0020 ppm)
GD	0.044 mg/m^3 (0.0057 ppm)	0.025 mg/m^3 (0.0033 ppm)	0.018 mg/m^3 (0.0022 ppm)	0.0085 mg/m^3 (0.0012 ppm)	0.0065 mg/m^3 (0.00085 ppm)
GF	0.044 mg/m^3 (0.0062 ppm)	0.025 mg/m^3 (0.0035 ppm)	0.018 mg/m^3 (0.0024 ppm)	0.0085 mg/m^3 (0.0013 ppm)	0.0065 mg/m^3 (0.00091 ppm)

$C^2 \times t = k$;
$([0.125 \text{ mg/m}^3]/30)^2 \times 0.5 \text{ h} = k$;
$k = 8.7 \times 10^{-6} \text{ mg/m}^3 \times \text{h}$.

The resulting AEGL-2 values are summarized in Table 1–34. The calculations of exposure concentrations for humans scaled for all AEGL-2 time points are shown in Appendix A.

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

Human lethality data resulting from exposure to any of the G agents were not available for deriving an AEGL-3.

TABLE 1–34 AEGL-2 Valuesa for Agent VX (mg/m3 [ppm])

10 min	30 min	1 h	4 h	8 h
0.0072 mg/m^3 (0.00065 ppm)	0.0042 mg/m^3 (0.00038 ppm)	0.0029 mg/m^3 (0.00027 ppm)	0.0015 mg/m^3 (0.00014 ppm)	0.0010 mg/m^3 (0.000095 ppm)

[a]The AEGL values are for vapor exposures only.

7.2. Summary of Animal Data Relevant to AEGL-3

Agent GB

Data on the lethality of GB are available for several laboratory species (see Table 1–9). Mioduszewski et al. (2000, 2001, 2002a) reported LCt_{50} and LC_{50} values for rats for exposure time periods of 10, 30, 60, 240, and 360 min. Bide et al. (1999) (see also Yee et al. [1999]), determined LC_{50} values for mice for time periods of 1 s to 30 min and estimated LC_{50} values for five other laboratory species and humans using a three-dimensional probit model.

Agent GD

In an experimental exposure study designed to secondarily examine agent GD toxicity, Aas et al. (1985) reported that the LCt_{50} for GD in rats (six animals tested at each of three exposure levels for periods of time <30 min) was 400 mg·min/m^3. Aas et al. (1985) graphically present their data as an LCt-versus-mortality curve. As estimated from this curve, the lethality threshold for rats exposed to GD is about 335 mg·min/m^3. Because the reported GD air concentration was fixed at 21 mg/m^3, the exposure time corresponding to the threshold was back-calculated to equal 16 min.

Note that the principal objective of the Aas et al. (1985) study was to test an experimental dynamic flow system that would allow study of highly toxic vapors. Secondary objectives of the study were to determine the (short-term) inhalation toxicity of agent GD (soman) and to study inhibition of acetylcholinesterase, cholinesterase, and carboxylesterase activity in the respiratory tract (relative to other tissues).

Agent GF

A recent study of GF vapor inhalation toxicity in male and female SD rats reported 24-h postexposure LC_{50} values for three exposure periods (10, 60, and 240 min) (Anthony et al. 2002). Young adult rats were exposed whole-body in a dynamic 750-L chamber under protocols similar to those previously published by Mioduszewski et al. (2001, 2002a) but with additional accommodations for the lesser volatility of agent GF. For female rats, Anthony et al. (2002) report 24-h postexposure LC_{50} values as follows: 10 min, 25.3 mg/m^3; 60 min, 5.56 mg/m^3; 240 min, 2.22 mg/m^3. For male rats, 24-h postexposure LC_{50} values are as follows: 10 min, 36.8 mg/m^3; 60 min, 6.60 mg/m^3; 240 min, 2.48 mg/m^3. These results are summarized as LCt_{50} values in Table 1–16. The preliminary data of Anthony et al. (2002) document 24-h lethality and LC_{50} only (Table 1–16). In consequence, these data are not comparable to the 14-d postexposure rat LC_{01} information available from the Mioduszewski et al. studies for GB vapor inhalation lethality. Furthermore, the preliminary nature of the Anthony et al. (2002) documentation precludes LC_{01} determination by benchmark dose analysis at this time.

7.3. Derivation of AEGL-3 for Agents GB and GD

Agent GB

The most complete lethality data set for the relevant time periods is that presented by Mioduszewski et al. (2000, 2001, 2002a). The final report of this study (Mioduszewski et al. 2001, 2002a) is further documentation of the findings presented below. The acute lethal toxicity of GB to male and female Sprague-Dawley rats was evaluated for time periods of 10, 30, 60, 90, 240, and 360 min in a whole-body dynamic chamber. Ten males and 10 females were used for each concentration-time (Ct) combination, and 50 males and 50 females were used for each time point. GB concentrations ranged from about 2 mg/m^3 to 54 mg/m^3. Agent concentrations were confirmed in the exposure chamber by three procedures to allow point and continuous determinations (Mioduszewski et al. 2000, 2001, 2002a). Lethality was assessed at 24 h and at 14 d postexposure. Female rats were reported to be more sensitive to GB vapor toxicity than males over the range of exposure concentrations and durations studied. Please note that

comparison of LCt_{50} values for male and female rats exposed to vapor concentrations of GB from Mioduszewski et al. (2000, 2001, 2002a) and Callaway and Blackburn (1954) reports indicates that the range of ratios (F:M) is 0.54 to 0.80, with a geometric mean of 0.67 (see Table 1–25). Gender differences for lethality are reported by Mioduszewski et al. (2000, 2001, 2002a) to be statistically significant at $p<0.01$.

Probit analysis (MINITAB, version 13) presented in Mioduszewski et al. (2000) gave the following 14-d LC_{50} values for female rats exposed to agent GB vapor: 18.1 mg/m^3 for 10 min, 8.51 mg/m^3 for 30 min, 6.39 mg/m^3 for 60 min, 3.03 mg/m^3 for 4 h, and 2.63 mg/m^3 for 6 h. Based on a probit analysis of the data (Mioduszewski et al. 2000), the estimated LC_{01} values for the females are as follows: 11.537 mg/m^3 for 10 min, 5.836 mg/m^3 for 30 min, 4.006 mg/m^3 for 60 min, 2.087 mg/m^3 for 4 h, and 1.761 mg/m^3 for 6 h. Mioduszewski et al. (2000) note that these estimates of LC_{01} are associated with large error bars.

The AEGL-3 for agent GB was derived from the lethality data for female Sprague-Dawley rats Mioduszewski et al. (2000, 2001, 2002a).

Regarding selection of the species to be used in neurotoxicity tests, the EPA Health Effects Test Guidelines (OPPTS 870.6200, *Neurotoxicity Screening Battery*) published by the Office of Prevention, Pesticides and Toxic Substances (EPA 1998) state that "in general, the laboratory rat should be used." The experimental protocol of Mioduszewski et al. (2000, 2001, 2002a) followed the OPPTS guidelines concerning the species, age, gender, and number of animals per dose and control group. Furthermore, in their recent review of organophosphates insecticide toxicity data, Storm et al. (2000) consider rat organophosphate inhalation data to be a defensible basis for developing (occupational) exposure limits, especially in the absence of human exposure data.

As previously discussed in the text on AEGL-1 and AEGL-2 values, the recent miosis and lethality data of Mioduszewski et al. (2000, 2001, 2002a,b) are determined to be the best source of an estimate for the n value for GB response (see Appendix B). Therefore, n=2 is used as the scaling function for AEGL-3 derivations in the equation $(C^n \times t = k)$ according to the methods often Berge et al. (1986) to derive an 8-h AEGL-3 from the 6-h LC_{01}. All the other time-specific AEGL-3 values were derived directly from the LC_{01} values for female SD rats in the Mioduszewski et al. (2000) study.

Given that the AEGL-3 estimation for the G-series nerve agents is derived from a lethal inhalation toxicity study of adult female SD rats (Mioduszewski et al. 2000, 2001 2002a), it is reasonable to consider the

whole-organism response of lethality as an appropriate end point by which to compare data for rats (a CaE-rich species) with data for monkeys and dogs, two experimental species considered in earlier studies to possess no plasma carboxylesterase (Augustinsson 1959). Available experimental LCt_{50} data for the monkey, dog, and rat are presented in Table 1–13. As shown in Table 1–13, LCt_{50} values for 10-min exposures to GB are 310 mg·min/m^3 in mice, 181–226 mg·min/m^3 in rats, 60 mg·min/m^3 in dogs, and 74 mg·min/m^3 in monkeys. There is a 2- to 3-fold difference between rats and monkeys, and a 3- to 4-fold difference between rats and dogs. These comparisons indicate that, when challenged with a lethal concentration of GB vapor, adult female SD rats are more resistant than adult dogs or monkeys by approximate factors of 2 to 4. Species differences in carboxylesterase concentrations may account, in part, for these observed differences. Please see Section 4.5.2 for a more detailed discussion of carboxylesterases as detoxification enzymes for nerve agent exposures.

In the case of human lethality estimates, Bide et al. (1999) estimate GB inhalation toxicity values for humans by application of allometric model extrapolation from extensive experimental animal data. Their study estimates that a 2-min adult human LCt_{50} approximates 31 mg·min/m^3 (a 2-min LC_{50} of 15.5 mg/m^3). The resulting 2-min LC_{50} ratio with the female SD rat from Mioduszewski et al. (2000, 2001, 2002a) (2-min LCt_{50} of 104 mg/m^3 or 2-min LC_{50} at 52 mg/m^3) is

female SD rat:human (estimated)=52/15.5=3.4.

This comparison indicates that, when challenged with a lethal concentration of GB vapor, adult female SD rats (Mioduszewski et al. 2000, 2001) are likely to be more resistant than adult humans by a factor between 3.0 and 3.5.

The following summarizes the above analysis of interspecies UF for AEGL-3 estimates:

- The literature regarding carboxylesterase (CaE) in lab animals and humans indicates that CaE is present in human plasma as well as numerous other human tissues and organs (including those where exposure and distribution leading to death by G agent vapor toxicity would likely occur).
- Interspecies data for comparison of the whole-organism response of lethality indicates that, when challenged with a lethal concentration of GB vapor, adult female SD rats are more resistant than adult dogs or monkeys by approximate factors of 2 to 4. Species differences in carboxyl

esterase concentrations may account for these differences. Model predictions of human LCt_{50} indicate a rat:human ratio of between 3.0 and 3.5.

- The known detoxification potential of carboxylesterases is multifaceted and encompasses consideration of CaE amount, affinity, and inhibitor resistant esterase activity. The present state of incomplete characterization for human CaE precludes accurate prediction regarding CaE detoxification potential in a population of humans exposed to anticholinesterase compounds.

In conclusion, recent literature indicates that CaE detoxification potential exists in numerous human organs and tissue, including blood plasma. It is acknowledged that further experimental characterization of CaE detoxification potential in humans will be necessary before accurate prediction of the contributions CaE may make to human protection from anticholinesterase poisoning. Interspecies comparisons of lethality data for rats and monkeys (as well as estimated human LCt_{50} values) has been performed. The results indicate that an interspecies UF (rat-to-human) of approximately 3 for AEGL-3 determination is a reasonable characterization of the present state of knowledge for this parameter.

To accommodate known variation in human cholinesterase and carboxylesterase activity that may make some individuals susceptible to the effects of cholinesterase inhibitors such as nerve agents, a factor of 10 was applied for intraspecies variability (protection of susceptible populations). Because a modifying factor is not applicable for reasons previously outlined for AEGL-1 and AEGL-2, the composite UF for AEGL-3 determination for agent GB is equal to 30. From the experimental data, k was derived from the 6-h LC_{01} as

$([1.761\ mg/m^3]/30)^2 \times 6.0\ h = k$;
$k = 0.021\ mg/m^3 \times h$.

The resulting AEGL-3 estimates for agent GB are summarized in Table 1– 35.

Benchmark Exposure Analyses

A benchmark exposure calculation has been performed on the female rat 14-d vapor lethality data presented in the Mioduszewski et al. (2001)

report in accordance with guidance provided in the NRC standing operating procedures (NRC 2001, 45). For comparison, a NumberCruncher Statistical System analysis has also been completed.

TABLE 1–35 AEGL-3 Values for Agent GB (mg/m3 [ppm])

10 min	30 min	1 h	4 h	8 h
0.38 mg/m^3 (0.064 ppm)	0.19 mg/m^3 (0.032 ppm)	0.13 mg/m^3 (0.022 ppm)	0.070 mg/m^3 (0.012 ppm)	0.051 mg/m^3 (0.0087 ppm)

There appears to be some degree of controversy around using the BMD approach for acute lethality data. The SOP workgroup will address this issue in the future.

There are eight models that accept dichotomous data in the Benchmark Dose software package available on the EPA Web site (http://cfpub.epa.gov/ncea/cfm/bmds.cfm): gamma, logistic, log-log multistage, probit, log-probit, quantal-linear, quantal-quadratic, and Weibull. Evaluations were performed with all eight (multiplied by five time points, times the 5% response for the 95% Lower Confidence Limit (LCL) and the 1% Maximum Likelihood Estimate (MLE), as per the SOP). The Weibull and gamma programs would not run with the input data; contact with the EPA Webmaster eventually revealed, through systems testing, that these two models require entry of a zero-concentration effect value in order to converge. The Mioduszewski et al. (2001) data set does not contain any zero-concentration effects data, and its addition would be an artificial alteration of the data set. It was concluded that the content of the data set is not compatible with requirements of the Weibull and gamma models; thus no analyses of the vapor lethality data were performed with these two models.

Tables summarizing the statistical results of the Benchmark Exposure Concentration analysis are included in Appendix C. Table C-1 is a summary of LC_{01} values obtained from all the Benchmark Dose software routines; the ones on the lower tier of the table (logistic, multistage, quantal-linear, quantal-quadratic) are poor fits and are rejected from any further consideration. The first column following the exposure times is the set of MLE LC_{01} values used to develop the AEGL-3 estimates published in the *Federal Register* notice of May 2, 2001 (66 Fed. Reg. 21940 [2001]). The LC_{01} values in the second column following the exposure times are those published by Mioduszewski et al. (2001). All remaining values presented in Table C-1 were based on the raw experimental data presented in Mioduszewski et al. (2001).

The MINITAB log probit seems to be a reasonable fit with the lethality data, and the experimental results on which this analysis is based are published in Mioduszewski et al. (2001, 2002a).

Because the statistical routines used to evaluate the data in Mioduszewski et al. (2000) differ slightly from those used in Mioduszewski et al. (2001), the LC_{01} values employed in developing interim AEGL-3 determinations also differ slightly. The resulting LC_{01} and AEGL-3 estimates developed with the same calculational approach—with the UF and *n* values applied in the AEGL-3 determinations presented earlier (see Appendix A)—are summarized in Tables C-1 and C-2 of Appendix C. The *Federal Register* interim values for AEGL-3 (see Table C-2) are consistently lower or equal to the Mioduszewski et al. (2001) log probit derived estimates, with the single exception of the 4-h value. In the case of the 4-h value, the NAC interim AEGL-3 (0.070 mg/m^3) is somewhat greater than the 4-h AEGL-3 estimate derived from the Mioduszewski et al. (2001) log probit derivation (0.059 mg/m^3; see Table C-2), by 0.011 mg/m^3. The variation is slight.

The LC_{01} values presented in Mioduszewski et al. (2001), although slightly different from the preliminary results considered (Mioduszewski et al. 2000), represent a better documented and more widely accessible data set. These differences are acknowledged.

Agent GD

A relative potency approach is used to estimate AEGL-3 values for agent GD, and a discussion of the relative potency of agent GD and GB is provided in Section 4.3. The lethal potency of agent GD is considered equivalent to that of agent GB (see Table 1–22).

A secondary and short-term GD inhalation study of rat lethality for exposure times ≤30 min (Aas et al. 1985) lends support to the assumption of lethal equipotency for agents GB and GD when used as a secondary study for derivation of 10-min and 30-min AEGL-3 values and as a comparison with the values derived by the relative potency method. Aas et al. (1985) calculated an LCt_{50} of 400 $mg \cdot min/m^3$ for GB and graphically presented their data as an LCt-versus-mortality curve. As estimated from this curve, the lethality threshold for rats exposed to agent GD (six animals tested at each of three exposure levels for periods of time <30 min) is about 335 $mg \cdot min/m^3$. Because the GD air concentration was fixed at 21 mg/m^3,

the exposure time corresponding to the threshold could be back-calculated, and was found to be 16 min. This lethality threshold was used to derive a comparative estimate of the AEGL-3.

Regression analysis of the data of Aas et al. (1985) was not possible from the information provided. Because the principal mode of action (cholinesterase inhibition) for the G agents is identical, n=2 was used for deriving comparative AEGL-3 values from the GD data of Aas and his colleagues. Because of the sparse data set for GD, the full default values for interspecies (10) and intraspecies (10) uncertainty were applied. Because a modifying factor is not applicable, a composite UF of 100 was used in deriving comparative 10-min AEGL-3 and 30-min AEGL-3 estimates for agent GD from the data provided by Aas et al. (1985).

AEGL-3 values for exposure times different from the experimental times were scaled using an n of 2. From the experimental data, k was derived as

$([21\ mg/m^3]/100)^2 \times (16/60)\ h = k;$
$k = 0.012\ mg/m^3 \times h.$

The resulting comparative AEGL-3 estimates include a 10-min AEGL-3 estimate of 0.27 mg/m^3 and a 30-min AEGL-3 estimate of 0.15 mg/m^3. Details of the comparative derivation are provided in Appendix A.

The values derived from the Aas et. al (1985) study are in good agreement with those derived by means of relative potency comparison with agent GB for the same time periods.

The recommended AEGL-3 value estimates are those derived by relative potency comparison with agent GB (with agent GD being considered equipotent to agent GB for lethal effects), and are summarized in Table 1– 36 below.

7.4. Derivation of AEGL-3 Values for Agents GA and GF

A relative potency approach is used to estimate AEGL-3 values for agents GA and GF. A discussion of the relative potency of these agents to cause lethality is given in Section 4.3 and summarized in Table 1–22. The lethal potency of GA is considered to be one-half that of GB, and agent GF is considered to be equipotent to GB for lethality (Table 1–37). The preliminary GF lethality report of Anthony et al. (2002) is not sufficiently com

plete (no documentation of threshold lethality) to support an independent AEGL-3 estimation for agent GF (see Section 7.2).

TABLE 1–36 AEGL-3 Values for Agent GD (mg/m3 [ppm])

10-min	30-min	1-h	4-h	8-h
0.38 mg/m^3 (0.049 ppm)	0.19 mg/m^3 (0.025 ppm)	0.13 mg/m^3 (0.017 ppm)	0.070 mg/m^3 (0.0091 ppm)	0.051 mg/m^3 (0.0066 ppm)

7.5. Derivation of AEGL-3 Values for Agent VX

Credible data on the acute vapor exposure lethality of agent VX are available for only two laboratory species, mice and goats. The LCt_{50} values are 4.0 mg·min/m^3 for mice and 9.2 mg·min/m^3 for goats for 10-min exposures (Koon et al. 1960). However, LC_{01} estimates cannot be derived from the Koon et al. study, and the analytical methods employed in measurement of experimental VX concentrations are not considered acceptable by modern standards.

Because of inadequacies in the human and animal toxicological database for agent VX, the AEGL-3 for agent VX is derived from the AEGL-3 for agent GB using a relative potency approach. The AEGL-3 for agent GB was derived from the lethality data for female Sprague-Dawley rats (Mioduszewski et al. 2000, 2001, 2002a), as discussed more fully in Section 7.3.

Probit analysis (MINITAB, version 13) presented in Mioduszewski et al. (2000) gave the following 14-day LC_{50} values for female rats exposed to agent GB vapor: 18.1 mg/m^3 for 10 min, 8.51 mg/m^3 for 30 min, 6.39 mg/m^3 for 60 min, 3.03 mg/m^3 for 4 h, and 2.63 mg/m^3 for 6 h.

Based on a probit analysis of the data (Mioduszewski et al. 2000), the estimated 14-day LC_{01} values for the females are as follows: 11.54 mg/m^3 for 10 min, 5.84 mg/m^3 for 30 min, 4.01 mg/m^3 for 60 min, 2.09 mg/m^3 for 4 h, and 1.76 mg/m^3 for 6 h.

By applying the relative potency of 4 as described earlier, the estimated 14-day LC_{01} for female SD rats exposed to VX vapor are as follows: 2.89 mg/m^3 for 10 min, 1.46 mg/m^3 for 30 min, 1.00 mg/m^3 for 60 min, 0.52 mg/m^3 for 4 h, 0.44 mg/m^3 for 6 h.

TABLE 1–37 AEGL-3 Values for Agents GA and GF (mg/m3 [ppm])

Agent	10 min	30 min	1 h	4 h	8 h
GA	0.76 mg/m^3 (0.11 ppm)	0.38 mg/m^3 (0.057 ppm)	0.26 mg/m^3 (0.039 ppm)	0.14 mg/m^3 (0.021 ppm)	0.10 mg/m^3 (0.015 ppm)
GF	0.38 mg/m^3 (0.053 ppm)	0.19 mg/m^3 (0.027 ppm)	0.13 mg/m^3 (0.018 ppm)	0.070 mg/m^3 (0.0098 ppm)	0.051 mg/m^3 (0.0071 ppm)

The derived LC_{01} values above were adjusted by a total UF of 100. The use of a rat data set resulted in selection of an interspecies UF of 3; the full default value of 10 was not considered appropriate for the interspecies UF because the mechanism of toxicity in both laboratory rodents and humans is cholinesterase inhibition, and the experimental results of Maxwell (1992) indicate that endogenous carboxylesterases in rats confer no protection against lethal exposures of nerve agent VX. To accommodate known variation in human cholinesterase activity, the full default value of 10 for intraspecies uncertainty was considered necessary to protect susceptible populations. With the additional application of a modifying factor of 3 for the sparse VX data set, the total UF for AEGL-3 determination for agent VX is equal to 100.

To derive an AEGL-3 for a time periods not included with the experimental protocol of Mioduszewski et al. (2000, 2001, 2002a) (i.e., the 8-h AEGL), the 6-h data were scaled using the relationship $C^n \times t = k$ (ten Berge et al. 1986). An n value has not been determined experimentally for VX. However, because the mechanism of action (cholinesterase inhibition) is the same as that for agent GB, the n value of 2 used in the derivation of the AEGL values for GB is appropriate for deriving AEGL values for VX. The experimentally derived n=2 from the Mioduszewski et al. (2000, 2001, 2002a,b) rat miosis and lethality data sets for agent GB are used here as the scaling function for the agent-VX 8-h AEGL-3 values, rather than a default value. Therefore, using the 6-h estimated LC_{01},

$C^2 \times 6\text{ h} = k$;
$([0.44\text{ mg/m}^3]/100)^2 \times 6\text{ h} = k$;
$k = 1.16 \times 10^{-4}\text{ mg/m}^3 \times \text{h}$.

The resulting AEGL-3 values are summarized in Table 1–38. The calculations of exposure concentrations for humans scaled for all AEGL-3 time points are shown in Appendix A.

8. SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity End Points

A summary of the AEGLs for agents GA, GB, GD, GF and VX is shown in Table 1–39.

G-Series Agents

In consultation with experimental investigators at Porton Down (United Kingdom) and the TNO Prins Maurits Laboratory (Netherlands), the current analysis has determined that the miotogenic response of mammalian eyes to agent GB vapor exposure is similar across species. The species evaluated include standard laboratory animals (rabbits, rats, guinea pigs), nonhuman primates (marmosets), and humans. In consequence, the interspecies UF for the critical AEGL-1 end point of miosis is considered equal to 1. To accommodate known variation in human cholinesterase and carboxylesterase activity that may make some individuals susceptible to the effects of cholinesterase inhibitors such as nerve agents, a factor of 10 was applied for intraspecies variability (protection of susceptible populations). A modifying factor is not applicable. Thus, the total UF for estimating AEGL-1 values for agent GB is 10.

For the development of AEGL-2 values, the database for toxicological effects in humans is more complete for agent GB than for any of the other G agents. Sufficient human data are available to directly derive AEGL-2 values for agent GB. The toxicity end points used to derive the value were considered to be appropriate, and the lowest of the available exposure concentrations was used. The data were limited, however, by the maximum time of exposure of 30 min (Baker and Sedgwick 1996). The AEGL-1 and AEGL-2 values for agents GA, GD, and GF were derived from the AEGL-1 and AEGL-2 values for GB using the relative potency approach based on the potency of the agents to induce miosis. Agents GA and GB were considered to have an equivalent potency for causing miosis. Agents GD and

GF were considered equipotent for AEGL-1 and AEGL-2 effects, and twice as potent as agent GB. The use of these relative potency measures for the derivation of AEGL-2 values for GA, GD, and GF was a conservative approach compared with an estimate based on a fraction (one-third) of the AEGL-3 concentration level (NRC 2001), which would have resulted in higher AEGL-2 values for GA, GD, and GF.

TABLE 1–38 AEGL-3 Valuesa for Agent VX (mg/m3 [ppm])

10 min	30 min	1 h	4 h	8 h
0.029 mg/m^3 (0.0027 ppm)	0.015 mg/m^3 (0.0014 ppm)	0.010 mg/m^3 (0.00091 ppm)	0.0052 mg/m^3 (0.00048 ppm)	0.0038 mg/m^3 (0.00035 ppm)

[a]The AEGL values are for vapor exposures only.

Lethality data for SD rats were available for the derivation of an AEGL-3 for agent GB. The AEGL-3 values for agents GA, GD, and GF were derived from the AEGL-3 values for GB using a relative potency approach. Agents GB, GD, and GF were considered to have an equivalent lethal potency. The lethal potency of agent GA was considered to be only one-half that of agents GB, GD, and GF.

A secondary derivation of 10-min and 30-min AEGL-3 values for agent GD relied on data from an inhalation study of male Wistar rats exposed to GD for periods up to 30 min (Aas et. al. 1985). The values derived from this secondary study (0.27 mg/m^3 for 10 min and 0.15 mg/m^3 for 30 min) are in good agreement with those derived by means of relative potency comparison with agent GB (0.38 mg/m^3 for 10 min and 0.19 mg/m^3 for 30 min) for the same time periods.

Cross-comparison of the AEGL estimates for agent GB with the available human toxicity data (Figure 1–1; see also Tables 1–7, 1–8, and 1–9) reveals that the AEGL-1 values for agent GB are substantially below any reported effect levels, and the AEGL-2 values are below any of the exposure levels that might be considered disabling. Although available human data are clustered in time periods <60 min, it is clear that AEGL-3 estimates are below exposures considered capable of inducing reversible AEGL-2 effects (miosis, dyspnea, changes in SFEMG, etc.) (Baker and Sedgewick 1996) and overlap those where reversible discomfort is observed. Comparisons with animal toxicity data (Figure 1–2) also indicate that the estimated AEGL values for GB are below most reported effect

TABLE 1–39 Relational Comparison of AEGLs for Nerve Agentsa GA, GB, GD, GF, and VX

Agent	Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
GA	AEGL-1 (Nondisabling)	0.0010 ppm (0.0069 mg/m^3)	0.00060 ppm (0.0040 mg/m^3)	0.00042 ppm (0.0028 mg/m^3)	0.00021 ppm (0.0014 mg/m^3)	0.00015 ppm (0.0010 mg/m^3)	Based on relative potency from GB[b]
	AEGL-2 (Disabling)	0.013 ppm (0.087 mg/m^3)	0.0075 ppm (0.050 mg/m^3)	0.0053 ppm (0.035 mg/m^3)	0.0026 ppm (0.017 mg/m^3)	0.0020 ppm (0.013 mg/m^3)	Based on relative potency from GB[b]
	AEGL-3 (Lethal)	0.11 ppm (0.76 mg/m^3)	0.057 ppm (0.38 mg/m^3)	0.039 ppm (0.26 mg/m^3)	0.021 ppm (0.14 mg/m^3)	0.015 ppm (0.10 mg/m^3)	Based on relative potency from GB[c]
GB	AEGL-1 (Nondisabling)	0.0012 ppm (0.0069 mg/m^3)	0.00068 ppm (0.0040 mg/m^3)	0.00048 ppm (0.0028 mg/m^3)	0.00024 ppm (0.0014 mg/m^3)	0.00017 ppm (0.0010 mg/m^3)	EC_{50} for miosis observed in adult female SD rats exposed to a range of GB vapor concentrations (0.01 to 0.48 mg/m^3) for 10, 60 and 240 min (Mioduszewski et al. 2002b) AND miosis data from secondary and supportive studies with

marmosets (van Helden et al. 2001, 2002), and humans (Harvey 1952; and Johns 1952).						
AEGL-2 (Disabling)	0.015 ppm	0.0085 ppm	0.0060 ppm	0.0029 ppm	0.0022 ppm	Miosis, dyspnea, RBC-ChE inhibition, single fibre electromyography (SFEMG) changes in human volunteers exposed to 0.5 mg/m^3 for 30 min (Baker and Sedgwick 1996)
AEGL-3 (Lethal)	(0.087 mg/m^3) 0.064 ppm (0.38 mg/m^3)	(0.050 mg/m^3) 0.032 ppm (0.19 mg/m^3)	(0.035 mg/m^3) 0.022 ppm (0.13 mg/m^3)	(0.017 mg/m^3) 0.012 ppm (0.070 mg/m^3)	(0.013 mg/m^3) 0.0087 ppm (0.051 mg/m^3)	Based on experimental SD rat lethality data (LC_{01} and LC_{50}); whole-body dynamic exposure to concentrations between 2–54 mg/m^3 for 3, 10, 30, 60, 90, 240, and 360 min

Agent	Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
	(Mioduszewski et al. 2000; 2001, 2002a)						
GD	AEGL-1 (Nondisabling)	0.00046 ppm (0.0035 mg/m^3)	0.00026 ppm (0.0020 mg/m^3)	0.00018 ppm (0.0014 mg/m^3)	0.000091 ppm (0.00070 mg/m^3)	0.000065 ppm (0.00050 mg/m^3)	Based on relative potency from GB[d]
	AEGL-2 (Disabling)	0.0057 ppm (0.044 mg/m^3)	0.0033 ppm (0.025 mg/m^3)	0.0022 ppm (0.018 mg/m^3)	0.0012 ppm (0.0085 mg/m^3)	0.00085 ppm (0.0065 mg/m^3)	Based on relative potency from GB[d]
	AEGL-3 (Lethal)	0.049 ppm (0.38 mg/m^3)	0.025 ppm (0.19 mg/m^3)	0.017 ppm (0.13 mg/m^3)	0.0091 ppm (0.070 mg/m^3)	0.0066 ppm (0.051 mg/m^3)	Based on relative potency from GB. Supported by Wistar rat LC_{50}; dynamic chamber exposures at 21 mg/m^3 for 3 time periods of ≤30 min duration (Aas et al. 1985)[e]
GF	AEGL-1 (Nondisabling)	0.00049 ppm (0.0035 mg/m^3)	0.00028 ppm (0.0020 mg/m^3)	0.00020 ppm (0.0014 mg/m^3)	0.00010 ppm (0.00070 mg/m^3)	0.000070 ppm (0.00050	Based on relative potency from GB[d]

						mg/m^3)	
	AEGL-2 (Disabling)	0.0062 ppm (0.044 mg/m^3)	0.0035 ppm (0.025 mg/m^3)	0.0024 ppm (0.018 mg/m^3)	0.0013 ppm (0.0085 mg/m^3)	0.00091 ppm (0.0065 mg/m^3)	Based on relative potency from GB[d]
	AEGL-3 (Lethal)	0.053 ppm (0.38 mg/m^3)	0.027 ppm (0.19 mg/m^3)	0.018 ppm (0.13 mg/m^3)	0.0098 ppm (0.070 mg/m^3)	0.0071 ppm (0.051 mg/m^3)	Based on relative potency from GB[e]
VX[f]	AEGL-1 (Non-disabling)	0.000052 ppm (0.00057 mg/m^3)	0.000030 ppm (0.00033 mg/m^3)	0.000016 ppm (0.00017 mg/m^3)	0.0000091 ppm (0.00010 mg/m^3)	0.0000065 ppm (0.000071 mg/m^3)	Derived by relative potency from EC_{50} for miosis observed in adult female SD rats exposed to a range of GB vapor concentrations (0.01 to 0.48 mg/m^3) for 10, 60 and 240 min (Mioduszewski et al. 2002b) AND miosis data from secondary and supportive studies of van Helden et al (2001,

Agent	Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
	2002), Harvey (1952) and Johns (1952) in marmosets and humans, respectively[g]						
	AEGL-2 (Disabling)	0.00065 ppm (0.0072 mg/m^3)	0.00038 ppm (0.0042 mg/m^3)	0.00027 ppm (0.0029 mg/m^3)	0.00014 ppm (0.0015 mg/m^3)	0.000095 ppm (0.0010 mg/m^3)	Derived by relative potency from study ofGB vapor exposure to exercising human volunteers exposed to 0.5 mg/m^3 for 30 min; miosis, dyspnea, inhibition of RBC-ChE changes in single fibre electromyography (SFEMG) (Baker and Sedgwick 1996)[h]
	AEGL-3 (Lethal)	0.0027 ppm (0.029	0.0014 ppm (0.015	0.00091 ppm (0.010	0.00048 ppm (0.0052	0.00035 ppm (0.0038	Derived by relative potency from

mg/m^3)	mg/m^3)	mg/m^3)	mg/m^3)	mg/m^3)	experimental SD rat lethality data (LC_{01})[f] and LC_{50}); whole-body dynamic exposure to GB vapor concentrations between 2–54 mg/m^3 for 3, 10, 30, 60, 90, 240, and 360 min (Mioduszewski et al. 2000; 2001; 2002a

[a]The derived AEGL values are for vapor exposures only. Percutaneous absorption of nerve agent vapors is known to be an effective route of exposure; nevertheless, percutaneous vapor concentrations needed to produce similar adverse effects are greater than inhalation vapor concentrations by several orders of magnitude (for agent VX, the percutaneous vapor concentrations needed to produce similar adverse effects are greater than inhalation vapor concentrations by an approximate factor of 10). Thus, the AEGL values presented are considered protective for both inhalation and percutaneous routes of exposure.

[b]Based on relative potency equal to that of agent GB (see Section 4.3 and Mioduszewski et al. [1998]).

[c]Agent GA is considered approximately one-half as potent as GB in causing lethality; thus, AEGL-3 values for GA are estimated by multiplying each time-specific AEGL-3 value for agent GB by a factor of 2 (see Section 4.3 and Mioduszewski et al. [1998]).

[d]Agents GD and GF are considered approximately twice as potent as agents GA and GB for causing miosis, and equipotent to each other. Thus, AEGL-1 and AEGL-2 values are estimated by multiplying each time-specific AEGL-1 or AEGL-2 value for agent GB by a factor of 0.5 (see Section 4.3 and Mioduszewski et al. [1998]).

[e]Based on a relative potency for lethality of GD=GF=GB and lethality data of Aas et al. (1985) (which provides a 10-min

AEGL-3 estimate of 0.27 mg/m^3 and a 30-min AEGL-3 value of 0.15mg/m^3 and is thus supportive of the GD AEGL-3 estimates derived from relative potency) (see Section 4.3 and Appendix A).

[f]Based on relative potency. Agent VX is considered approximately 4 times more potent than agent GB. (See Section 4.3.4, Grob and Harvey [1958], and Sidell and Groff [1974].)

[g]Derived from miosis effects noted in young adult female SD rats exposed to agent GB vapor at concentrations (0.010 to 0.48 mg/m^3) for 10, 60 and 240 min (Mioduszewski et al. 2002b). VX concentration to achieve same end point estimated by relative potency adjustment presented in footnote *f* above.

[h]Derived from transient effects noted in exercising human volunteers exposed to agent GB vapor at 0.5 mg·min/m^3 for 30 min (Baker and Sedgwick 1996). VX concentration to achieve same end point estimated by relative potency adjustment presented in footnote *f* above.

[i]Derived from LC_{01} values for female Sprague-Dawley rats exposed to GB vapor in dynamic exposure chamber (Mioduszewski et al. 2000, 2001, 2002a). VX concentrations to achieve same end point estimated by relative potency adjustment presented in footnote *f* above.

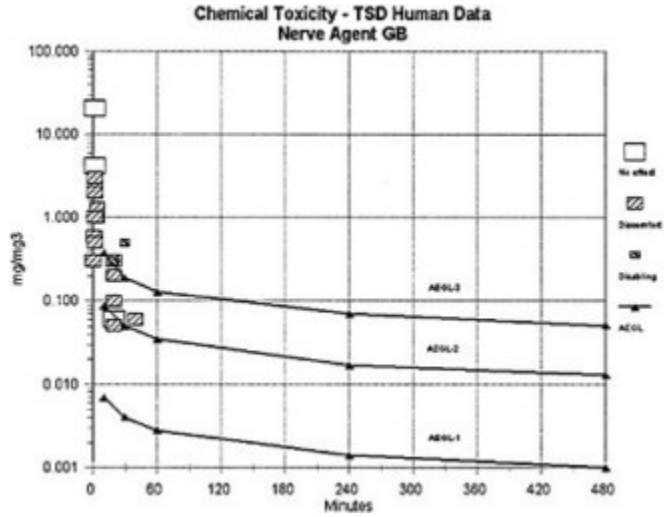


FIGURE 1–1 Comparison of AEGL values for agent GB with human toxicity data.

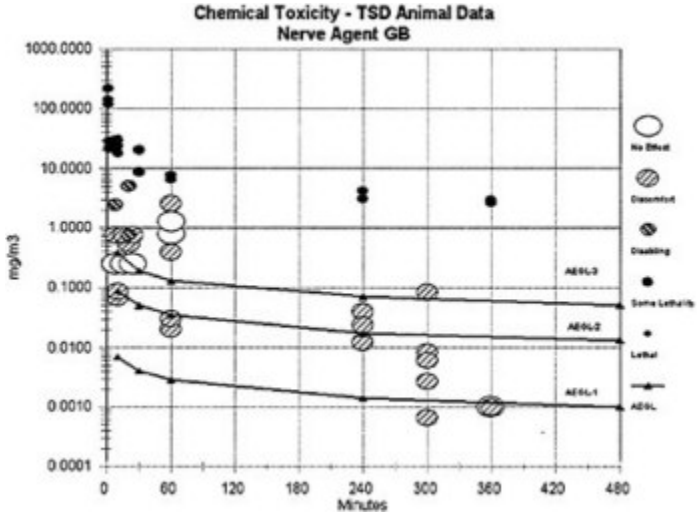


FIGURE 1–2 Comparison of AEGL values for agent GB with animal toxicity data.

levels. Estimates of AEGL-3 for GB for time periods <60 min overlap those considered to cause no effect or discomfort. Therefore, the AEGL estimates for agent GB are considered protective. Although comparative data for the other G agents are quite limited, the animal toxicity data that are available for agent GD and GF (Figures 1–3 and 1–4) further support the conclusion that the current AEGL estimates are protective for all levels of effect.

Agent VX

For the development of AEGL values for Agent VX, toxicity end points specific for each of the three AEGL levels were not available. Therefore, each of the AEGL values was derived from the corresponding critical AEGL study for agent GB, using the relative potency approach and the assumption that the potency of VX was 4 times greater than that of GB. After relative potency adjustment, the 8-h AEGL-3 for VX was scaled from the 6-h experimental data point for GB (see Appendix A).

Cross-comparison of the AEGL estimates for agent VX with available human toxicity data (Figure 1–5) reveals that the AEGL-3 estimates for agent VX are well below reported human exposure levels (median of 3.6 mg/m^3) that result in mild, reversible discomfort (headache, chest tightness, and dryness of the mouth) (Koon et al. 1959). Lethal exposures in animals (Figure 1–6) occur 1 to 2 orders of magnitude above AEGL-3 estimates.

These comparisons with available experimental data indicate that the final AEGL estimates for agent VX are protective.

8.2. Comparison with other Standards and Criteria

G Agents

Exposure guidelines for the nerve agents GA, GB, and GD have been established by several organizations. All currently available guidelines are shown in Tables 1–40, 1–41, and 1–42. No comparable values have been established for exposure to agent GF. Values were not found for the following standards and guidelines: ERPGs, PELs, RELs, TLVs, and MAKs.

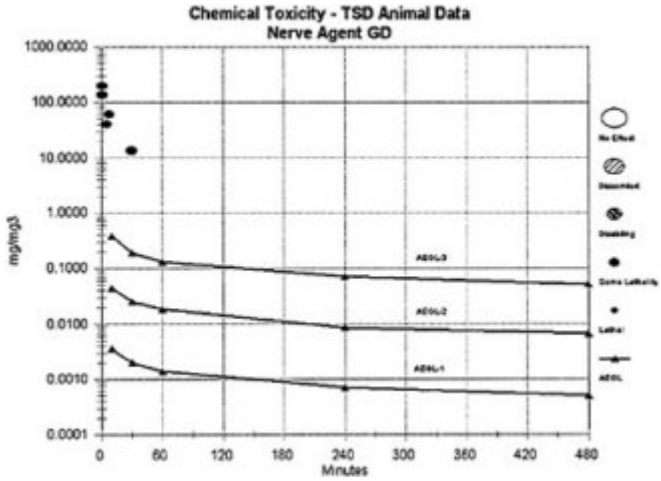


FIGURE 1–3 Comparison of AEGL values for agent GD with animal toxicity data.

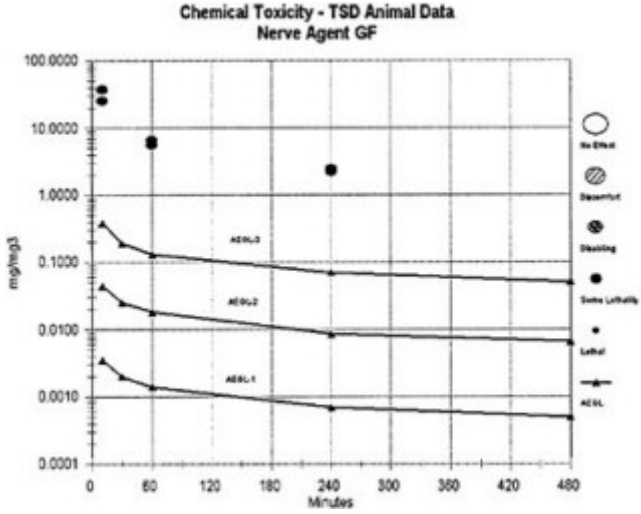


FIGURE 1–4 Comparison of AEGL values for agent GF with animal toxicity data.

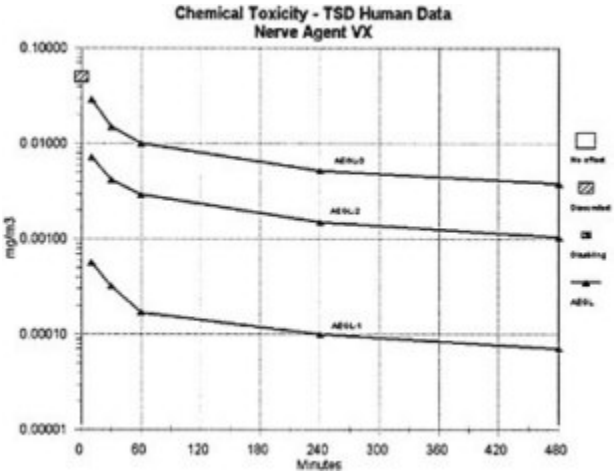


FIGURE 1–5 Comparison of AEGL values for agent VX (RP=4) with human toxicity data.

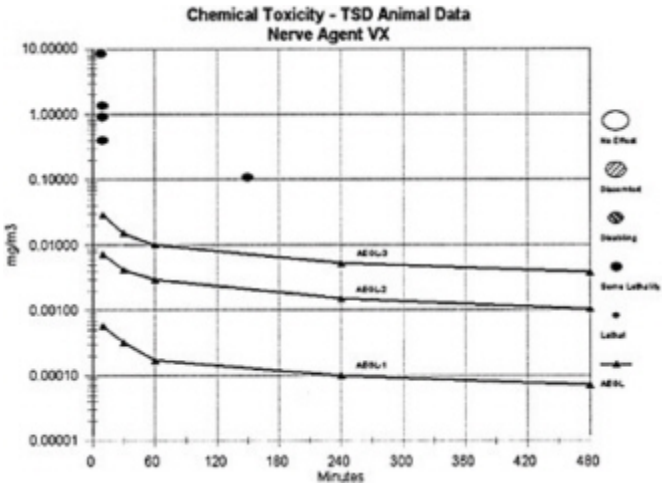


FIGURE 1–6 Comparison of AEGL values for agent VX (RP=4) with animal toxicity data.

TABLE 1–40 Extant Standards and Guidelines for Nerve Agent GA (mg/m3)a

Guideline	10 min	30 min	1 h	4 h	8 h	24 h
AEGL-1[b]	0.0069	0.0040	0.0028	0.0014	0.0010	
AEGL-2[b]	0.087	0.050	0.035	0.017	0.013	
AEGL-3[b]	0.76	0.38	0.26	0.14	0.10	
Army IDLH[c]		0.2				
DHHS TWA[d]					0.0001	0.000003

[a]Existing exposure guidelines for nerve agents are published in mg/m^3; the guidelines are presented here as they exist in the literature and have not been converted to parts per million.
[b]Percutaneous absorption of G agent vapor is known to be an effective route of exposure; nevertheless, percutaneous vapor concentrations needed to produce similar adverse effects are greater than inhalation vapor concentrations by several orders of magnitude. Thus, the AEGL values presented are considered protective for both routes of exposure.
[c]DA 1997.
[d]DHHS 1988; 8-h value is TWA for occupational exposure; 24-h value is TWA for the general population.

At present, the only chemical warfare exposure limits published in the United States for use in civilian community emergency preparedness planning are those developed by the Department of Health and Human Services (DHHS 1988; Thacker 1994). For the agents GA and GB, the current time-weighted average (TWA) to be applied as a no-adverse-health-effect level for 24-h continuous exposure to the general population is 3×10^{-6} mg/m^3. For these same agents, the 8-h TWA to be applied as a no-adverse-health-effect level for 8-h continuous workplace exposure for worker populations is 1×10^{-4} mg/m^3 (DHHS 1988). Agents GD and GF, which are not part of the unitary stockpile, were not evaluated by DHHS in 1988. As part of a regularly scheduled review process, the Centers for Disease Control and Prevention (CDC) is currently reevaluating the 1988 agent control limits with application of recent risk assessment models and updated scientific data (67 Fed. Reg. 895 [2002]; DHHS 2002). This review is currently (September 2002) in progress, and the CDC has not yet released a final position.

TABLE 1–41 Extant Standards and Guidelines for Nerve Agent GBa

Guideline	No Time Period (Ct) (mg·min/m^3)	10-min (mg/m^3)	30-min (mg/m^3)	1-h (mg/m^3)	4-h (mg/m^3)	8-h (mg/m^3)	24-h (mg/m^3)
AEGL-1[b]		0.0069	0.0040	0.0028	0.0014	0.0010	
AEGL-2[b]		0.087	0.050	0.035	0.017	0.013	
AEGL-3[b]		0.38	0.19	0.13	0.070	0.051	
Army IDLH[c]			0.2				
DHHS TWA[d]						0.0001	0.000003
DHHS ATEL[e]	0.5						

[a]Existing exposure guidelines for nerve agents are published in mg/m^3; the guidelines are presented here as they exist in the literature and have not been converted to parts per million.
[b]Percutaneous absorption of G agent vapor is known to be an effective route of exposure; nevertheless, percutaneous vapor concentrations needed to produce similar adverse effects are greater than inhalation vapor concentrations by several orders of magnitude. Thus, the AEGL values presented are considered protective for both routes of exposure.
[c]DA 1997.
[d]DHHS 1988; 8-h value is TWA for occupational exposure; 24-h value is TWA for the general population
[e]Acute Threshold Effects level (ATEL); Thacker 1994; based on linear extrapolation equivalent to 10 min to 0.05 mg/m^3, 30 min to 0.017 mg/m^3, 1 h to 0.0083 mg/m^3, 4 h to 0.0021 mg/m^3, or 8 h to 0.0010 mg/m^3.

TABLE 1–42 Extant Standards and Guidelines for Nerve Agent GD (mg/m3)a

Guideline	10 min	30 min	1 h	4 h	8 h	24 h
AEGL-1[b]	0.0035	0.0020	0.0014	0.00070	0.00050	
AEGL-2[b]	0.044	0.025	0.018	0.0085	0.0065	
AEGL-3[b]	0.38	0.19	0.13	0.070	0.051	
Army IDLH[c]		0.06				
Army TWA[c]					0.00003	0.000003

[a]Existing exposure guidelines for nerve agents are published in mg/m^3; the guidelines are presented here as they exist in the literature and have not been converted to parts per million.
[b]Percutaneous absorption of G agent vapor is known to be an effective route of exposure; nevertheless, percutaneous vapor concentrations needed to produce similar adverse effects are greater than inhalation vapor concentrations by several orders of magnitude. Thus, the AEGL values presented are considered protective for both routes of exposure.
[c]DA 1997.

The CDC has also established an acute threshold effects level (ATEL) for agent GB (Thacker 1994). An ATEL is a cumulative exposure (Ct) (concentration in mg/m^3 multiplied by time in minutes [mg·min/m^3]; Ct does not express the amount retained within the organism [Sidell 1997]). These ATELs are considered by CDC to represent "lowest-observed-effect-levels" that "could be exceeded without danger" to the public and form the basis for planning protective actions, such as emergency evacuations, in the Chemical Stockpile Emergency Preparedness Program (CSEPP) of the Federal Emergency Management Agency and the Department of the Army. The Acute Threshold Effect Levels are described by CDC as protective of the general population (including consideration of vulnerable subgroups such as infants, the elderly, and debilitated or ill persons) (Thacker 1994). The ATEL for agent GB is 0.5 mg·min/m^3, a protective cumulative exposure at which miosis is not expected to occur in humans (McNamara and Leitnaker 1971). If projected GB concentrations resulting from a release event were to result in Cts >0.5 mg·min/m^3, then the CDC would consider

protective measures (such as evacuation or shelter-in-place) warranted as a means of providing maximal protection to safeguard the general public. The CDC did not designate the exposure time periods applicable to the ATEL.

For comparison to the AEGLs for the G agents, the CDC ATEL for GB can be converted (by direct linear extrapolation) to the following time-specific agent concentrations: 10-min, 0.05 mg/m^3; 30-min, 0.017 mg/m^3; 1-h, 0.0083 mg/m^3; 4-h, 0.0021 mg/m^3; or 8-h, 0.0010 mg/m^3. These estimated concentrations are all greater than the agent-GB AEGL-1 values (with the exception of the 8-h interval, for which the ATEL and the AEGL-1 values are nominally equal) and less than the agent-GB AEGL-2 values. The estimated AEGL-1 and AEGL-2 values are thus in keeping with the CDC ATEL for agent GB (Thacker 1994).

Acute threshold effects levels have not been established for agents GA, GD, and GF.

Agent VX

Exposure guidelines for the nerve agent VX have been established by several organizations. All currently available guidelines are shown in Table 1–43.

At present, the only chemical warfare agent exposure limits published in the United States for use in civilian community emergency preparedness planning are those developed by the Department of Health and Human Services (DHHS 1988; Thacker 1994). For agent VX, the current time-weighted average (TWA) to be applied as a no-adverse-health-effect level for 24-h continuous exposure to the general population is 3×10^{-6} mg/m^3. The 8-h TWA to be applied as a no-adverse-health-effect level for 8-h continuous workplace exposure for worker populations is 1×10^{-5} mg/m^3 (DHHS 1988). As stated earlier, this review is currently (September 2002) in progress, and the CDC has not yet released a final position.

ATELs have also been developed by the CDC for agent VX (Thacker 1994). The ATEL value for agent VX is 0.4 mg·min/m^3; the CDC did not designate specific exposure time periods applicable to the ATEL. If projected VX concentrations resulting from a release event resulted in VX Cts >0.4 mg·min/m^3, the CDC would consider protective measures (such as evacuation or shelter-in-place) warranted as a means of providing maximal protection to safeguard the general public.

TABLE 1–43 Extant Standards and Guidelines for Nerve Agent VX[a]

Guideline	No Time Period (Ct) (mg·min/m^3)	10 min (mg/m^3)	30 min (mg/m^3)	1 h (mg/m^3)	4 h (mg/m^3)	8 h (mg/m^3)	24 h (mg/m^3)
AEGL-1[b]		0.00057	0.00033	0.00017	0.00010	0.000071	
AEGL-2[b]		0.0072	0.0042	0.0029	0.0015	0.0010	
AEGL-3[b]		0.029	0.015	0.010	0.0052	0.0038	
Army IDLH[c]			0.02				
DHHS TWA[d]						0.00001	0.000003
DHHS ATEL[e]	0.4						

[a]Existing exposure guidelines for nerve agents are published in mg/m^3; the guidelines are presented here as they exist in the literature, and have not been converted to parts per million.
[b]These final values are for vapor exposures only.
[c]DA 1997.
[d]DHHS 1988; 8-h value is TWA for occupational exposure, 5 d/wk; 24-h value is TWA for the general population, 7 d/wk.
[e]Thacker 1994.

For comparison to AEGLs for VX, the CDC ATEL for VX can be converted by extrapolation to the following time-specific agent concentrations: 10-min, 0.04 mg/m^3; 30-min, 0.013 mg/m^3; 1-h, 0.0067 mg/m^3; 4-h, 0.0017 mg/m^3; or 8-h, 0.00083 mg/m^3. These values are in excess of AEGL-2 estimates for VX exposure durations ≤1 h. They are approximately equivalent to AEGL-2 estimates for VX exposure durations ≥4 h.

Because the VX AEGLs are derived in this report by comparison to the AEGL for agent GB, it should be noted that the Acute Threshold Effects Level for agent GB is 0.5 mg·min/m^3.

8.3. Data Adequacy and Research Needs

G Agents

Confidence in the AEGL-2 values is limited by the sparse human experimental data for all AEGL-specific time frames and G-series agents. The human data from which the AEGL-2 values were derived were limited by short exposure times (30 min). The AEGL-1 values for agent GB derived from rat data (Mioduszewski et al. 2002b) are supported by 5-h exposure studies conducted on marmosets (van Helden et al. 2001, 2002) and 20-min exposure studies conducted with human volunteers (Harvey 1952; Johns 1952).

Observed differences among studies in identifying the toxicity threshold for low-dose exposures may be due to differences in individual sensitivities and breathing rates among the test populations, the steepness of the dose-response curve, and/or differences in experimental protocols or analytical methods. These variables need further characterization.

Further data analysis and experimentation are needed to more fully understand the degree of susceptibility to lethal exposure exhibited by female populations of test animals. Interspecies susceptibility could be more fully characterized by determining if similar results can be obtained under the same protocol with different test species (particularly nonhuman primates).

The scarcity of dose-response data for agents GA, GD, and GF forces the AEGL analysis to rely on assumptions of relative potency. Thus, AEGL values for these three compounds are largely derivative (with the exception of 10-min and 30-min AEGL-3 values for agent GD, which are confirmed by short-term experimental lethality data for Wistar rats provided by Aas et. al. [1985]).

The relative potency assumptions for estimating AEGL-1, AEGL-2, and AEGL-3 values for agents GA, GD, and GF from the available database for agent GB need experimental confirmation.

Additional research is needed to compare and contrast effects of all nerve agents on noncholinergic neurotransmitters and neurotransmitter receptor sites for correlating reported effects observed in rat hippocampal cells in vitro (see Section 4.2) with whole-organism responses to acutely toxic dose levels. Extrapolation of findings from such neurophysiological research to whole organisms may allow a more refined quantitative analysis of the nerve agent relative potency.

Agent VX

The available experimental human acute toxicity data for agent VX are limited by the short exposure times (7 min or less), uncontrolled atmospheres for inhalation studies, inadequate exposure protocols, or inappropriate exposure routes. Longer-term inhalation exposure data would be useful for deriving more reliable AEGL-1 values. The Bramwell et al. (1963) study is a flawed and suspect source and could not be applied in deriving exposure estimates.

Only a single nonlethal animal inhalation toxicity study has been conducted on agent VX (Crook et al. 1983). The results of this animal study are considered nonverifiable and are thus not adequate for deriving an AEGL-1 or an AEGL-2 for VX.

Credible acute animal lethality data for inhalation vapor exposures to agent VX are available only in the form of LCt_{50} values for exposure times of 10 min or less for two species, mice and goats (Koon et al. 1960, as cited in NRC 1997). Available animal lethality data for VX do not cover the time periods relevant for deriving AEGL-3 values. A comprehensive animal lethality data set is needed for directly deriving AEGL-3 estimates.

Confidence in the AEGL values derived for agent VX is limited by sparse human inhalation data for the AEGL-specific time frames and a definitive assessment of the exposure-response relationship for humans exposed to VX vapor. Derivation of AEGL-1, AEGL-2, and AEGL-3 values using the relative potency method and comparison with agent GB is limited by uncertainties associated with the derivation of the values for agent GB as well as uncertainties associated with estimating the potency of VX relative to that of GB.

Further experimentation is needed to more fully understand the degree of sensitivity to lethal exposure exhibited by female populations of test animals. Interspecies sensitivity could be more fully characterized by determining if similar results can be obtained with different test species (particularly nonhuman primates).

It is noted that specific experimental focus should be put on obtaining data that would reduce uncertainties regarding the relative potency of agents GB and VX, or the potency of agent VX, for critical effects such as miosis, rhinorrhea, and lethality; such studies could be adequately performed on a limited test population and scale. Tests should be performed with a single species (preferably laboratory rat), both genders, and should involve vapor exposures over the range of 10 min to 8 h (minimally, for 10 min, 1 h, and 6 h). It is further recommended that dosages selected begin slightly below the recommended AEGL-1 estimate and increase in 10-fold increments to include the anticipated LCt_{50}. Clinical effects should be observed, and histopathology and necropsy performed on a sample from each Ct group.

In addition, research characterizing the emission profile of vapor and aerosols expected during VX release events is needed for estimation of potential differential exposure and toxicity. Agent VX parameters needing quantification by modern methods and protocols include: generation and yield of vapors versus aerosols; particle size ranges; atmospheric half-times; deposition rates; and rates of degradation in air under various states of humidity, temperature, UV, et cetera. Until these parameters can be more fully characterized to inform the discussion of differential toxicity between VX vapors and aerosols, AEGL determinations will be necessarily based on the assumption of exposure to VX vapor.

9. REFERENCES

Aas, P., T.A.Veiteberg, and F.Fonnum, F. 1987. Acute and sub-acute inhalation of an organophosphate induced alteration of cholinergic muscarinic receptors. Biochem. Pharmacol. 36:1261–1266.

Aas, P., S.H.Sterri, H.P.Hjermstad, and F.Fonnum. 1985. A method for generating toxic vapors of soman: Toxicity of soman by inhalation in rats. Toxicol. Appl. Pharmacol. 80:437–445.

Abou-Donia, M.B. 1993. The cytoskeleton as a target for organophosphorous ester-induced delayed neurotoxicity (OPIDN). Chem.-Biol. Interactions 87: 383–393.

Abou-Donia, M.B., and D.M.Lapadula. 1990. Mechanisms of organophosphorous ester-induced delayed neurotoxicity: Type I and type II. Ann. Rev. Pharmacol. Toxicol. 30:405–440.

Abu-Qare, A.W., and M.B.Abou-Donia. 2001. Combined exposure to sarin and pyridostigmine bromide increased levels of rat urinary 3-nitrotyrosine and 8-hydroxy-2'-deoxyguanosine, biomarkers of oxidative stress. Toxicol. Lett. 123:51–58.

ACGIH (American Conference of Governmental Industrial Hygienists). 1996. Supplements to the Sixth Edition; Documentation of the Threshold Limit Values and Biological Exposure Indices. American Conference of Governmental Industrial Hygienists, Cincinnati, OH. (pp. 1–7 for n-butyl acetate).

ACGIH (American Conference of Governmental Industrial Hygienists). 2002. 2002 TLVs and BEIs; Threshold Limit Values and Biological Exposure Indices for chemical substances and physical agents. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

A.D. Little, Inc. 1985. Pharmacokinetic parameters of selected organophosphate compounds with anticholinesterase activity. AAMRL-TR-85–040 (DTIC AD-A157 923). Prepared by Arthur D. Little, Inc., Cambridge, MA, for Air Force Aerospace Medical Research Laboratory, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson Air Force Base, Ohio 45433.

Adams, N.L., J.von Bredow, and H.V.deVera. 1975. Intramuscular Lethality of GD (Soman) in the Rhesus monkey. EB-TR-76039. Biomedical Laboratory, Edgewood Arsenal, MD.

Allebrandt, K.V., R.L.R.Souza, and E.A.Chautard-Freire-Maia. 2002. Variability of the paraoxonase gene (PON1) in Euro- and Afro-Brazilians. Toxicol. Appl. Pharm. 180:151–156.

Allon, N., L.Raveh, E.Gilat, E.Cohen, J.Grunwald, and Y.Ashani. 1998. Prophylaxis against soman inhalation toxicity in guinea pigs by pretreatment alone with human serum butyrylcholinesterase. Toxicol. Sci. 43:121–128.

AMRL (Aerospace Medical Research Laboratory). 1977. Proceedings of the Annual Conference on Environmental Toxicology (7th). Report No. AMRL-TR-76–125. Aerospace Medical Research Laboratory, Wright-Patterson, Air Force Base, OH, Oct. 13–15, 1976.

Ammon, R. 1933. The enzymatic hydrolysis of acetylcholine. Arch. Ges. Physiol. 233:486.

Anthony, J.S., M.V.Haley, J.H.Manthei, R.A.Way, D.C.Burnett, B.P.Gaviola, D.R.Sommerville, R.B.Crosier, R.J.Mioduszewski, E.M.Jakubowski, J.L. Montgomery, and S.A.Thomson, S.A. 2002. Inhalation toxicity of GF vapor in rats as a function of exposure concentration and duration and its potency comparison to GB. Late-breaking poster presented at 41st Annual Meeting of the Society of Toxicology, Nashville, TN (21 Mar 2002).

Antikainen, M., S.Murtomaki, M.Syvanne, R.Pahlman, E.Tahvanainen, M. Jauhiainen, M.H.Frick, and C.Ehnholm. 1996. The Gln-Arg 191 polymor

phism of the human paraoxonase gene (HUMPONA) is not associated with the risk of coronary artery disease in Finns. J. Clin. Invest. 98:883–885.

Anzueto, A., R.A.DeLemos, J.Seidenfeld, G.Moore, H.Hamil, D.Johnson, and S.G.Jenkinson. 1990. Acute inhalation toxicity of soman and sarin in baboons. Fund. Appl. Toxicol. 14:676–687.

Araneta, M.R., C.A.Moore, R.S.Olney, L.D.Edmonds, J.A.Karcher, C. McDonough, K.M.Hiliopoulos, K.M.Schlangen, and G.C.Gray. 1997. Goldenhar syndrome among infants born in military hospitals to Gulf War veterans. Teratology 56:244–251.

Ashani, Y., J.Grunwald, L.Raveh, et al. 1993. Cholinesterase prophylaxis against organophosphorous poisoning. AD-A277096. U.S. Army Medical Research, Development, Acquisition and Logistics Command, Ft. Derrick, MD.

Ashwick, W.E., and C.A.deCandole. 1961. A Further Trial of Atropine, Metaraminol, and P2S in the Treatment of GB Poisoning in the Monkey. Suffield Technical Paper No. 208. Defence Research Board, Department of National Defence, Canada.

Atchison, C.R., C.Holmes, S.Akers, et al. 2001. Guinea pig model for low dose chronic exposure to nerve agent. Proceedings of the Scientific Conference on Chemical and Biological Defense Research. March 6–8, 2001, Hunt Valley, MD.

Augustinsson, K.-B. 1955. The normal variation of human blood cholinesterase activity. Acta Physiol. Scand. 35:40–52. (As cited in Hayes 1982.)

Augustinsson, K.-B. 1959. Electrophoresis studies on blood plasma esterases. I. Mammalian plasmata. Acta. Chem. Scand. 13:571–592.

Baker, D.J., and E.M.Sedgwick. 1996. Single fibre electromyographic changes in man after organophosphate exposure. Hum. Exp. Toxicol. 15:369–375.

Bakerman, S. 1984. ABC's of Interpretive Laboratory Data, 2nd Ed. Interpretative Laboratory Data, P.O. Box 7066, Greenville, NC (p. 127).

Bakry, N.M., A.H.El-Rashidy, A.T.Eldefawi, and M.E.Eldefawi. 1988. Direct action of organophosphate anticholinesterases on nicotinic and muscarinic acetylcholine receptors. J. Biochem. Toxicol. 3:235–259.

Bakshi, K.S., S.N.J.Pang, and R.Snyder, eds. 2000. Review of the U.S. Army's Health Risk Assessments for oral exposure to six chemical-warfare agents. J. Toxicol. Environ. Health (Part A):59(5–6):281–526.

Bates, H.K., J.B.LaBorde, J.C, Dacre, and J.F.Young. 1990. Developmental toxicity of soman in rats and rabbits. Teratology 42:15–23.

Benke, G.M., and S.D.Murphy. 1975. The influence of age on the toxicity and metabolism of methyl parathion and parathion in male and female rats. Tox. Appl. Pharmac. 31:254–269.

Benschop, H.P. 1999. Toxicokinetics of O-Ethyl S-(2-Diisopropylaminoethyl) Methylphosphonothioate [(±)−VX] in Hairless Guinea Pigs and Marmosets-Identification of Metabolic Pathways. Annual Report (Project: DAMD17–97– 2–7001). Annual Report (Project: DAMD17–97–2–7001). Medical Research and Materiel Command, Fort Derrick, MD.

Benschop, H.P., C.A.G.Konings, and L.P.A.de Jong. 1981. Gas chromatographic separation and identification of the four stereoisomers of 1,2,2-trimethylpropyl methylphosphonofluoridate (soman). Stereospecificity of in vitro "detoxification" reactions. J. Am. Chem. Soc. 103:4260–4262.

Benschop, H.P., H.C.Trap, H.E.T.Spruit, et al. 1998. Low-level nose-only exposure to the nerve agent soman: toxicokinetics of soman stereoisomers and cholinesterase inhibition in atropinized guinea pigs. Toxicol. Appl. Pharmacol. 153:179–185.

Benschop, H.P., M.J.van der Schans, and J.P.Langenberg. 2000. Toxicokinetics of O-Ethyl S-(2-Diisopropylaminoethyl)Methylphosphonothioate [(±)−VX] in Rats, Hairless Guinea Pigs and Marmosets—Identification of Metabolic Pathways. Final Report (Project: DAMD17–97–2–7001). Prepared for U.S. Army Medical Research and Materiel Command, Fort Detrick, MD.

Berteau, P.E., and R.E.Chiles. 1978. Studies on the inhalation toxicity of two phosphoramidothiate insecticides to rodents and quail. Toxicol. Appl. Pharmacol. 45:232.

Bide, R., J.Armour, and E.Yee. 1999. An approach to obtain estimates of human toxicity. Part III: A reasonable, defendable procedure to obtain human inhalation toxicity estimates (LCt05, LCt_{50} and LCt_{95}) directly from animal toxicity data. Presented to the NATO Challenge Subgroup, 12–14 May, 1999, San Antonio, TX.

Bide, R.W., and D.J.Risk. 2000. Inhalation Toxicity of Aerosolized Nerve Agents. 1. VX Revisited. Technical Report DRES TR 2000–063. Defence Research Establishment Suffield, Alberta, Canada.

Bide, R.W., and D.J.Risk. 2001. GB toxicity in mice exposed for 20 min to 720 min (U). DRES-TR-2000–173. Defence Research Establishment Suffield, Ralston, Alberta, Canada (in review).

Billecke, S.S., S.L.Primo-Parmo, C.S.Dunlop, J.A.Doorn, B.N.LaDu, and C.A. Broomfield. 1999. Characterization of a soluble mouse liver enzyme capable of hydrolyzing diisopropyl phosphoroflouridate. Chemico-Biological Interactions 119–120:251–256.

Bonderman, R.P., and D.P.Bonderman. 1971. Atypical and inhibited human serum pseudocholinesterase: A titrimetric method for differentiation. Arch. Environ. Health 22:578–581.

Boter, H.L., A.J.J.Ooms, G.R.Van DenBerg, and C.Van Dijk. 1966. The synthesis of optically active isopropyl methylphosphonofluoridate (sarin). Recueil 85:147–150.

Bowers, M.B., E.Goodman, and V.M.Sim. 1964. Some behavioral changes in man following anticholinesterase administration. J. Nerv. Mental Dis. 138:383–389.

Bramwell, E.C.B., W.S.S.Ladell, and R.J.Shephard. 1963. Human exposure to VX vapour. Porton Technical Paper No. 830. Porton Down, Salisbury, Wiltshire, UK.

Britton, K.B., and C.L.Grant. 1988. Prediction of octanol-water partition coeffi

cients of organophosphates. Evaluation of structure-function relationships. Special Report 88–11, U.S. Department of the Army, Corps of Engineers Cold Regions Research and Engineering Laboratory, Hanover, NF.

Brown, M.A., and K.A.Brix. 1998. Review of health consequences from high-, intermediate- and low-level exposure to organophosphorus nerve agents. J. Appl. Toxicol. 18:393–408.

Bucci, T.J. and R.M.Parker. 1992. Toxicity studies on agents GB and GD (phase II): 90-day subchronic study of GB (sarin type II) in CD rats. Final report (DTIC: AD-A248618). E515 and E516. National Center for Toxicological Research. Prepared for the U.S. Department of the Army, Biomedical Research and Development Laboratory, Ft. Detrick, MD.

Bucci, T.J., R.M.Parker, J.A.Crowell, J.D.Thurman and P.A.Gosnell. 1991. Toxicity studies on agents GB and GD (phase II): 90-day subchronic study of GB (sarin type I) in CD rats. FDA 224–85–0007 (DTIC: AD-A248617). Prepared for the U.S. Department of the Army, Biomedical Research and Development Laboratory, Ft. Detrick, MD.

Bucci, T.J., R.M.Parker, J.A.Crowell, J.D.Thurman, and P.A.Gosnell. 1992. Toxicity studies on agent GA (phase II): 90-day subchronic study of GA (tabun) in CD rats. Final report (DTIC: AD-A258020). Prepared for the U.S. Department of the Army, Biomedical Research and Development Laboratory, Ft. Detrick, MD.

Bucci, T.J., J.D.Fikes, R.M.Parker, K.H.Denny, and J.C.Dacre. 1993. Developmental toxicity study (segment II: teratology) of tabun in CD rats and New Zealand white rabbits. Prepared for the U.S. Department of the Army, Medical Research and Development Command, Ft. Detrick, MD.

Burchfiel, J.L., and F.H.Duffy. 1982. Organophosphate neurotoxicity: Chronic effects of sarin on the electroencephalogram of monkey and man. Neurobehav. Toxicol. Teratol. 4:767–778.

Burnett, W.T., and J.E.Chambers. 1994. Age-related toxicity of the insecticides parathion and chlorpyrifos. Toxicologist 14:390. (As cited in Davies 1996.)

California Environmental Protection Agency. 1998. Medical Supervision. Pesticide and Safety Information, Series A, No. HS-8. Worker Health and Safety Branch, California Environmental Protection Agency, Department of Pesticide Regulation, Sacramento, CA (Sept 20, 1998).

Callaway, S., and J.W.Blackburn. 1954. A comparative assessment of the vapour toxicities of GB, GD, GF, T-2132, T-2137, and T-2146 to male and female rats. Porton Technical Paper No. 404. Chemical Defence Establishment, Porton Down, England.

Callaway, S., and D.Crichton. 1954. The production of casualties in monkeys with GB vapor. Porton Technical Paper No. 424. Chemical Defence Establishment, Porton Down, England.

Callaway S., and P.Dirnhuber. 1971. Estimation of the concentration of nerve agent vapour required to produce measured degrees of miosis in rabbit and

human eyes. Technical Paper No. 64. Chemical Defence Establishment, Porton Down, Salisbury, Wiltshire, UK.

Carnes, S.A. 1989. Disposing of the chemical weapons: A desired end in search of an acceptable means. Environ. Prof. 11(4):279–290.

Carnes, S.A., J.A.Boyette, F.C.Kornegay, et al. 1989. Preliminary Assessment of the health and environmental impacts of incinerating M55 rockets stored at Pine Bluff Arsenal, Lexington-Blue Grass Depot Activity, and/or Anniston Army Depot and Pine Bluff Arsenal, ORNL 6197, Oak Ridge National Laboratory, Oak Ridge TN.

Carnes, S.A., and A.P.Watson. 1989. Disposing of the U.S. chemical weapons stockpile: An approaching reality. JAMA 262:653–659.

Carroll, N.C., F.C.G.Hoskin, M.K.McPhail, and D.K.Myers. 1957. Vapour toxicity of VE and VX to female mice. STP 121. Suffield Experimental Station, Canada.

Cashman, J.R., B.Y.Perotti, C.E.Berkman, and J.Lin. 1996. Pharmacokinetics and molecular detoxication. Environ. Health Perspect. 104:23–40.

Chanda, S.M., T.L.Lassiter, V.C.Moser, S.Barone, Jr., and S.Padilla. 2002. Tissue carboxylesterases and chlorpyrifos toxicity in the developing rat. HERA 8:75–90.

Chebabo, S.R., M.D.Santos, and E.X.Albuquerque. 1999. The organophosphate sarin, at low concentrations, inhibits the evoked release of GABA in rat hippocampal slices. Neurotoxicol. 20(6):871–882.

Chen, S.-M., and M.-G.Chi. 1986. Direct effect of VX and soman on nicotinic receptors. Acta Pharmacol. Sinica 7:401–406.

Christen, P.J., and J.A.C.M.van den Muysenberg. 1965. The enzymatic isolation and fluoride catalyzed racemisation of optically active sarin. Biochim. Biophys. Acta. 110:217–220.

Christensen, M.K., P.Cresthull, and F.W.Oberst. 1958. ICt_{99} and LCt_{1-99} estimates of GB Vapor for man at various exposure times. Chemical Warfare Laboratories Report 2266, Army Chemical Center, MD.

Ciliberto, C.F., and G.F.Marx. 1998. Physiological changes associated with pregnancy. Physiology 9 (2) [Online]. Available at www.nda.ox.ac.uk/wfsa/html/u09/u09_005.htm [accessed on January 28, 2002].

Clark, D.N. 1989. Review of reactions of chemical agents in water. DTIC: AD-A213 287. Defense Technical Information Center, Alexandria, VA.

Clement, J.G. 1983. Effect of pretreatment with sodium phenobarbitol on the toxicity of soman in mice. Biochem. Pharmacol. 32:1411–1415.

Clement, J.G. 1984. Role of aliesterase in organophosphate poisoning. Fund. Appl. Toxicol. 4:S96–S105.

Clement, J.G. 1992. Efficacy of various oximes against GF (Cyclohexylmethylphosphonofluoridate) poisoning in mice. Arch. Toxicol. 66:143–144.

Clement, J.G. 1994. Toxicity of the combined nerve agents GB/GF in mice: Efficacy of atropine and various oximes as antidotes. Arch. Toxicol. 68:64–66.

Cohen, B.M., P.J.Christen, and E.Mobach. 1971. The inactivation by oximes of sarin and soman in plasma from various species. I. The influence of diacetylmonoxime on the hydrolysis of sarin. J.A.Cohen Memorial Issue, Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen (Proceedings of the Royal Netherlands Academy of Sciences and Letters). North-Holland, Amsterdam, pp. 113–131.

Cohen, B.S., F.W.Oberst, J.W.Crook, and C.Harris. 1954. Effect of repeated exposures of GB vapor on erythrocyte and brain cholinesterase in rats. Medical Laboratories Research Report No. 297. Army Chemical Center, MD.

Conn, C.A., K.Dokladny, M.G.Ménache, E.B.Barr, W.Kozak, A.Kozak, M. Wachulec, K.Rudolph, M.J.Kluger, and R.F.Henderson. 2002. Effects of sarin on temperature and activity of rats as a model for Gulf War Syndrome neuroregulatory functions. Toxicol. Appl. Pharm. 184:77–81.

Cresthull, P., W.S.Koon, F.P.McGrath, and F.W.Oberst. 1957. Inhalation effects, incapacitation and mortality for monkeys exposed to GA, GB, and GF Vapors. Chemical Warfare Laboratory Report No. 2179. Army Chemical Center, MD.

Cresthull, P., W.S.Koon, and N.P.Musselman. 1963. Percutaneous exposure of the arm or forearm of man to VX vapor. U.S. Army Chemical Research and Developmental Laboratories, Technical Report No. 3176. Edgewood Arsenal, MD.

Cresthull, P., J.W.Crook, M.K.Christensen, et al. 1959. Relationship between the LD_{50} and ChE_{50} values for monkeys and dogs exposed to GB by the inhalation and intravenous routes. Chemical Warfare Laboratory Report No. 2280. Army Chemical Center, MD.

Crook, J.W., P.Hott, E.J.Owens, et al. 1983. The effects of subacute exposures of the mouse, rat, guinea pig, and rabbit, to low-level VX concentrations. U.S. Army Armament Research and Development Command, Chemical Systems Laboratory, Technical Report ARCSL-TR-82038, Aberdeen Proving Ground, MD.

DA (Department of the Army). 1974. Chemical agent data sheets, vol. 1. Edgewood Arsenal Special Report, EO-SR-74001. U.S. Department of the Army, Edgewood Arsenal, Aberdeen Proving Ground, MD.

DA (Department of the Army). 1987. Safety regulations for chemical agents H, HD, HT, GB and VX. AMC-R385–131. U.S. Department of the Army, Material Command, Alexandria, VA.

DA (Department of the Army). 1988. Final programmatic environmental impact statement for the chemical stockpile disposal program. U.S. Department of the Army, Office of the Program Executive Officer, Program Manager for Chemical Demilitarization, Aberdeen Proving Ground, MD.

DA (Department of the Army). 1990a. Occupational health guidelines for evaluation and control of occupational exposure to nerve agents GA, GB, GD and VX. DA Pam 40–8. U.S. Department of the Army, Headquarters. Washington, DC.

DA (Department of the Army). 1990b. Potential Military Chemical/Biological Agents and Compounds. Field Manual FM 3–9 (NAVFAC P-467, AFR 355– 7), Headquarters, Department of the Army, Department of the Navy, Department of the Air Force, Washington, DC (12 December 1990).

DA (Department of the Army). 1991. Occupational health guidelines for evaluation and control of occupational exposure to nerve agents GA, GB, GD and VX. DA Pam 40–8. U.S. Department of the Army, Headquarters, Washington, DC (Interim change I01, Revision, 15 May 91).

DA (Department of the Army). 1992. Material safety data sheets: Lethal nerve agents GA, GB, VX. U.S. Department of the Army, Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, MD.

DA (Department of the Army). 1997. The Army Chemical Agent Safety Program. Army Regulation 385–61 (AR 385–61). Headquarters, Department of the Army, Washington, DC. (26 Feb 1997).

DA (Department of the Army). 2001. Transportable treatment systems for nonstockpile chemical warfare materiel, Programmatic Environmental Impact Statement, Vol. I and II. U.S. Department of the Army, Project Manager for Non-Stockpile Chemical Materiel, Aberdeen Proving Ground, MD (February 2001).

Dacre, J.C. 1984. Toxicology of some anticholinesterases used as chemical warfare agents—a review. Pp. 415–426. in: Cholinesterases: Fundamental and Applied Aspects, M.Brzin, E.A.Barnard, and D.Sket, eds. New York: Walter de Gruyter.

Davies, H.G., R.J.Richter, M.Keifer, C.A.Broomfield, J.Sowalla, and C.E.Furlong. 1996. The effect of human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin. Nat. Genet. 14:334–336.

de Jong, L.P.A., and G.Z.Wolring. 1978. Effect of 1-(AR)alkyl-2-hydroxyiminomethyl-pyridinium salts on reactivation and aging of acetylcholinesterase inhibited by ethyl dimethylphosphor- amidocyanidate (tabun). Biochem. Pharmacol. 27:2229–2235.

de Wolff, F.A., N.Treijtel, and M.Vermeulen. 2002. Mechanisms of peripheral neurotoxicity. Chapter. 15, pp. 282–303, in Site-Selective Neurotoxicity, D.S. Lester, W.Slikker, and P.Lazarovici, eds. New York: Taylor and Francis Publishers.

Denk, J.R., 1975. Effects of GB on mammalian germ cells and reproductive performance. EB-TR-74087, U.S. Department of the Army, Edgewood Arsenal, Aberdeen Proving Ground, MD.

DHHS (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention). 1988. Final recommendations for protecting the health and safety against potential adverse effects of long-term exposure to low doses of agents GA, GB, VX, Mustard Agent (H, HD, T), and Lewisite (L). Fed. Reg. 53:8504–8507, 1988.

DHHS (Department of Health and Human Services). 2002. Airborne exposure

limits for chemical warfare agents GA (Tabun), GB (Sarin), and VX. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Fed. Reg. 67 (5):894–901 (8 January 2002).

Due, H.A., H.C.Trap, H.J.Van der Wiel, and H.P.Benschop. 1993. Effect of pretreatment with CBDP on the toxicokinetics of soman stereoisomers in rats and guinea pigs. Arch. Toxicol. 67:760–711.

Duffy, F.H., J.L.Burchfiel, P.H.Bartels, M.Gaon, and V.M.Sim. 1979. Long-term effects of an organophosphate upon the human electroencephalogram. Toxicol. Appl. Pharmacol. 47:161–176.

Dunn, M.A., and F.R.Sidell. 1989. Progress in the medical defense against nerve agents. J. Amer. Med. Assoc. 262:649–652.

Dutreau, C.W., F.P.McGrath, and E.H.Bray, Jr. 1950. Toxicity studies on GD. 1. Median lethal concentration by inhalation in pigeons, rabbits, rats, and mice. 2. Median detectable concentration by odor for man. MDRR 8, CMLEM-52. Chemical Corps, Medical Division, Army Chemical Center, MD (ADE 470029 058).

EGESAQ (Egezsegtudomany). 1980. 24:173 (Kultura, POB 149, H-1389, Budapest, Hungary).

Ehrich, M., and B.S.Jortner. 2002. Organophosphorous-induced delayed neuropathy. Chapter 2, pp. 17–25 in Handbook of Neurotoxicity, Vol. 1, E.J.Massaro, ed. Totowa, NJ: Humana Press.

Ellin, R.I. 1981. Anomalies in theories and therapy of intoxication by potent organophosphorus anticholinesterase compounds. U.S. Army Medical Research and Development Command, Biomedical Laboratory, Report No. USABML-SP-81–003. Aberdeen Proving Ground, MD. DTIC, ADA101364.

Ellman, G.L., K.D.Courney, V.Andres, Jr., and R.M.Featherstone. 1961. A new rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharm. 7:88–95.

EPA (U.S. Environmental Protection Agency). 1992. Draft report: A cross-species scaling factor for carcinogen risk assessment based on equivalence of $mg/kg^{3/4}$/day. Fed. Reg. 57(109):24152–24173.

EPA (U.S. Environmental Protection Agency). 1998. Health Effects Test Guidelines. OPPTS 870.6200 Neurotoxicity Screening Battery. Office of Prevention, Pesticides and Toxic Substances. EPA 712-C-98–238, U.S. Environmental Protection Agency, Washington, DC (August 1998).

EPA (U.S. Environmental Protection Agency). 2000. Office of Pesticide Programs science policy on the use of data on cholinesterase inhibition for risk assessment of organophosphorus and carbamate pesticides. Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, DC (August 18, 2000).

Eto, M. 1974. Organophosphorus Pesticides: Organic and Biological Chemistry. Cleveland, OH: CRC Press. Pp. 123–231.

Fairley, A., and S.A.Mumford. 1948. Detection of the G gases by smell. PTP 74. Porton Down, England.

FCH (Farm Chemicals Handbook). 1991. Farm Chemicals Handbook, Meister Publishing, Willoughy, OH.

FEMA/DA (Federal Emergency Management Agency/U.S. Department of the Army). 1996. Planning Guidance for the Chemical Stockpile Emergency Preparedness Program. Federal Emergency Management Agency and U.S. Department of the Army, Washington, DC.

Fogleman, R.W., J.P.Saunders, and R.T.Goring. 1954. The respiratory effects of inhaled toxic vapors dispersed in air. II. The subacute effects on dogs of low inhaled dosages of GB administered by the dosimetric technique. Medical Laboratory Research Report, MLRR 287. Army Chemical Center, MD.

Fonnum, F., and S.H.Sterri. 1981. Factors modifying the toxicity of organophosphorous compounds, including soman and sarin. Fund. Appl. Toxicol. 1:143–147.

Freeman, G., F.N.Marzulli, A.B.Craig, et al. 1954. The toxicity of liquid GA applied to the skin of man. Chemical Corps Medical Laboratory Research Report No. 250 (abstract only available).

Frostling, H. 1974. The vapour pressure of O-ethyl, S-3 (N,N-diisopropylamino)-ethylmethyl phosphonothiolate. Acta Chemica Scand. A28:83–85.

Furlong, C.E., R.J.Richter, S.L.Seidel, L.G.Costa, and A.G.Motulsky. 1988. Role of genetic polymorphism of human plasma paraoxonase/arylesterase in hydrolysis of the insecticide metabolites chlorpyrifos oxon and paraoxon. Am. J. Hum. Genet. 43:230–238.

Furlong, C.E., R.J.Richter, S.L.Seidel, L.G.Costa, and A.G.Motulsky. 1989. Spectrophotometric assays for the enzymatic hydrolysis of the active metabolites of chlorpyrifos and parathion by plasma paraoxonase/arylesterase. Analyt. Biochem. 180:242–247.

Furlong, C.E., W-F.Li, D.M.Shih, A.J.Lusis, R.J.Richter, and L.G.Costa. 2002. Genetic factors in susceptibility: Serum PON1 variation between individuals and species. HERA 8:31–43.

Goldman, M., L.S.Rosenblatt, B.W.Wilson, et al. 1988. Toxicity studies on agent VX: Final report. Laboratory for Energy-Related Health Research, University of California, Davis, CA.

Goldman, M., A.K.Klein, T.G.Kawakami, and L.S.Rosenblatt. 1987. Toxicity studies on agents GB and GD. DTIC: ADA187841. Laboratory for Energy-Related Health Research, Prepared for the U.S. Department of the Army, Medical Research and Development Command, Fort Derrick, MD.

Goldstein, G., S.R.Beers, and L.A.Morrow. 1996. A preliminary neuropsychological study of Persian Gulf veterans. J. Int. Neuropsychol. Soc. 2:368–371.

Gordon, J.J., R.H.Inns, M.K.Johnson, L.Leadbeater, M.P.Maidment, D.G.Upshall, G.H.Cooper, and R.L.Rickard. 1983. The delayed neuropathic effects of nerve agents and some other organophosphorous compounds. Arch. Toxicol. 52:71–82.

Grant, W.M. Toxicology of the Eye, Third Edition. 1986. Springfield, Illinois: Charles C.Thomas, Publisher.

Gray, G.C., B.D.Coate, C.M.Anderson, et al. 1996. The postwar hospitalization experience of U.S. veterans of the Persian Gulf War. New England J. Med. 335:1505–1513.

Gray, G.C., T.C.Smith, J.D.Knoke, and J.Heller. 1999. The postwar hospitalization experience of Gulf War veterans possibly exposed to chemical munitions destruction at Khamisiyah, Iraq. Amer. J. Epidemiol. 150:532–540.

Grob, D., and J.C.Harvey. 1958. Effects in man of the anticholinesterase compound Sarin (isopropyl methyl phosphonofluoridate). J. Clin. Invest. 37:350–368.

Gupta, R.C., G.T.Patterson, and W.-D.Dettbarn. 1987. Acute tabun toxicity: Biochemical and histochemical consequences in brain and skeletal muscles of rat. Toxicol. 46:329–341.

Gupta, R.C., G.T.Patterson, and W.-D.Dettbarn. 1991. Comparison of cholinergic and neuromuscular toxicity following acute exposure to sarin and VX in rat. Fund. Appl. Toxicol. 16:449–458.

Haboubi, N.A., and D.E.Thurnham. 1986. Effect of ethanol on erythrocyte acetylcholinesterase activity. Ann. Clin. Biochem. 23:458–462.

Haley, R.W. 1998. Counterpoint: Haley replies. Amer. J. Epidemiol. 148:334– 338.

Haley, R.W., and T.L.Kurt. 1997. Self-reported exposure to neurotoxic chemical combinations in the Gulf War: A cross-sectional epidemiologic study. J. Amer. Med. Assoc. 277:231–237.

Haley, R.W., T.L.Kurt, and J.Hom. 1997a. Is there a Gulf War Syndrome? Searching for syndromes by factor analysis of symptoms. J. Amer. Med. Assoc. 277:215–222.

Haley, R.W., J.Hom, and P.S.Roland. 1997b. Evaluation of neurologic function in Gulf War veterans: A blinded case-control study. J. Amer. Med. Assoc. 277:223–230.

Harris, H., and M.Whittaker. 1962. The serum cholinesterase variants: Study of twenty-two families selected via the “intermediate” phenotype. Ann. Hum. Genet. 26:59–72.

Harris, C., W.Koon, J.W.Christensen, and F.W.Oberst. 1953. Do pathological lesions develop in dogs repeatedly exposed to low concentrations of GB vapor? Medical Laboratory Research Report, MLRR 155. Army Chemical Center, MD.

Harris, L.W., W.C.Heyl, D.L.Stitcher, and C.A.Broomfield. 1978. Effects of 1,1-oxydimethylene-bis-(4-tert-butyl pyridinium chloride) (SAD-128) and decamethonium on reactivation of soman- and sarin-inhibited cholinesterase by oximes. Biochem. Pharmacol. 27:757–761.

Hartley, D., and H.Kidd, eds. 1983–1986. Agrochemicals Handbook. Nottingham, UK: Royal Soc. Chem.

Harvey, J.C. 1952. Clinical observations on volunteers exposed to concentrations of GB. Medical Laboratories Research Report No. 114, Publication Control No. 5030–114, MLCR 114 (CMLRE-ML-52). Army Chemical Center, MD.

Hayes, W.J. 1982. Pesticides Studied in Man. William and Wilkins, Baltimore, MD.

Henderson, R.F., E.B.Barr, W.B.Blackwell, C.R.Clark, C.A.Conn, R.Kalra, T.H.March, M.L.Sopori, Y.Tesfaigzi, M.G.Ménache, and D.C.Mash. 2002. Response of rats to low levels of sarin. Toxicol. Appl. Pharm. 184:67– 76.

Henderson, R.F., C.A.Conn, E.B.Barr, T.H.March, J.R.Krone, M.L.Sopori, Y. Tesfaigzi, M.Wachulec, and D.C.Mash. 2000. Effect of low level sarin exposure on physiological parameters in rats. Presented at the 39th Annual Meeting of the Society of Toxicity, March, 2000, Philadelphia, PA . Toxicologist 54(1):266 (#1248).

Henderson, R.F., E.B.Barr, C.R.Clark, M.L.Sopori, S.P.Singh, C.A.Conn, Y. Tesfaigzi, T.H.March, and D.C.Mash. 2001. Effects of inhalation exposure to low levels of sarin in Fischer 344 rats. Presented at the 40th Annual Meeting of the Society of Toxicity, March, 2001, San Francisco, CA (#1142).

Heston, W.E. 1942. Inheritance of susceptibility to spontaneous pulmonary tumors in mice. J. Natl. Cancer Inst. 3:79–82.

Himuro, K., S.Murayama, K.Nishiyama, et al. 1998. Distal axonopathy after sarin intoxication. Neurobiology 51:1195–1197.

Holmstedt, B. 1959. Pharmacology of organophosphorous cholinesterase inhibitors. Pharmacol. Rev. 11:567–688.

Hom, J., R.W.Haley, and T.L.Kurt. 1997. Neuropsychological correlates of Gulf War Syndrome. Arch. Clin. Neuropsychol. 12:531–544.

Husain, K., R.Vijayaraghavan, S.C.Pant, S.K.Raza, and K.S.Pandey. 1993. Delayed neurotoxic effect of Sarin in mice after repeated inhalation exposure. J. Appl. Toxicol. 13:1435.

Husain, K., S.C.Pant, S.K.Raza, R.Singh, and S.Das Gupta. 1995. A comparative study of delayed neuropathy in hens following repeated administration of organophosphorous compounds. Indian J. Physiol. Pharmacol. 39:47–50.

IOM (Institute of Medicine). 1999. Chemical and Biological Terrorism: Research and Development to Improve Civilian Medical Response. Committee on Research and Development Needs for Improving Civilian Medical Response to Chemical and Biological Terrorism Incidents, Institute of Medicine, Washington, DC: National Academy Press.

Ishiyama, I. 1996. Problems on the possibility in forensic diagnosis. Nippon Hoigaku Zasshi 50:220 (abstract).

Izmerov, N.F., I.V.Sanotsky, and K.K.Sidorov. 1982. Toxicometric Parameters of Industrial Toxic Chemicals under Single Exposure. USSR Commission for UNEP (United Nations Environmental Programme), Centre of International Projects, GKNT, Moscow.

Jacobson, K., M.K.Christensen, I.Dearmon, Jr., and F.W.Oberst. 1959. Studies on chronic exposures of dogs to GB vapor (isopropyl methylphosphonofluoridate). Arch. Ind. Health 19:5–10.

Johns, R.J. 1952. The effect of low concentrations of GB on the human eye.

Chemical Corps Medical Laboratories Research Report No. 100, Publication Control No. 5030–100 (CMLRE-ML-52). Army Chemical Center, MD.

Johnson, D.E., A.Anzueto, H.F.Hamil, J.H.Brewer, G.T.Moore, J.J.Siedenfeld, and R.A.Delemos. 1988. Studies of the effects of organophosphorus agent exposures on the lung. Southwest Research Institute, San Antonio, TX. Report prepared for the U.S. Army Medical Research and Development Command, Fort Detrick, MD. Defense Technical Information Center Report No. AD-A203861.

Jokanović, M. 1989. Role of carboxylesterase in soman, sarin and tabun poisoning in rats. Pharmacol. Toxicol. 65:181–184.

Jokanović, M., M.Kosanović, and M.Maksimović. 1996. Interaction of organophosphorus compounds with carboxylesterases in the rat. Arch. Toxicol. 70:444–450.

Jokanović, M., and M.Maksimović. 1997. Abnormal cholinesterase activity: Understanding and interpretation. Eur. J. Clin. Chem. Clin. Biochem. 35:11– 16 .

Jones, K.H., A.M.Dechkovskaia, E.A.Herrick, A.A.Abdel-Rahman, W.A.Khan, and M.B.Abou-Donia. 2000. Subchronic effects following a single sarin exposure on blood-brain and blood-testes barrier permeability, acetylcholinesterase, and acetylcholine receptors in the central nervous system of rat: A dose-response study. J. Toxicol. Environ. Health A 61:695–707.

Kadar, T., L.Raveh, and G.Cohen. 1985. Distribution of ^{3}H-Soman in mice. Arch. Toxicol. 58:45–49.

Kagan, I.U.S., G.A.Voitenko, T.N.Pan'shina, et al. 1983. Combined toxicological and hygienic study of the organophosphate insecto-acaricide actellic [in Russian]. Gig. Sanit. 1983(6):32–35.

Kaliste-Korhonen, E., K.Tuovinen., and O.Hanninen 1996. Interspecies differences in enzymes reacting with organophosphates and their inhibition by paraoxon in vitro. Hum. Exp. Toxicol. 15:972–980.

Kalra, R., S.P.Singh, S.Razani-Boroujerdi, R.J.Langley, W.B.Blackwell, R.F. Henderson, and M.L.Sopori. 2002. Subclinical doses of the nerve gas sarin impair T-cell responses through the autonomic nervous system. Toxicol. Appl. Pharm. 184:82–87.

Kang, H.K., and T.A.Bullman. 1996. Mortality among U.S. veterans of the Persian Gulf War. New England J. Med. 335:1498–1504.

Kassa, J., M.Pecka, M.Tich , J.Bajgar, M.Koupilová, J.Herink, and Z. Kročová. 2001. Toxic effects of sarin in rats at three months following single or repeated low-level inhalation exposure. Pharmacol. Toxicol. 88:209–212.

Kassa, J., and J.Cabal. 1999. A comparison of the efficacy of acetylcholinesterase reactivates against cyclohexyl methylphosphonofluoridate (GF Agent) by in vitro and in vivo methods. Pharmacol. Toxicol. 84:41–45.

Kato, T., and T.Hamanaka. 1996. Ocular signs and symptoms caused by exposure to sarin gas. Amer. J. Opthalmol. 121:209–210.

Kaufman, K. 1954. Serum cholinesterase activity in the normal individual and in people with liver disease. Ann. Intern. Med. 41:533–545.

Khan, W.A., A.Dechkovskaia, K.H.Jones, et al. 2000a. Acute sarin exposure leads to cholinergic dysregulation in central nervous system of rats. Presented at the 39th Annual Meeting of the Society of Toxicity, March, 2000, Philadelphia, PA. Toxicologist 54(1):265 (#1245).

Khan, W.A., A.M.Dechkovskaia, E.A.Herrick, K.H.Jones, and M.B.Abou-Donia. 2000b. Acute sarin exposure causes differential regulation of choline acetyltransferase, acetylcholinesterase, and acetylcholine receptors in the central nervous system of the rat. Toxicol. Sci. 57(1):112–120.

Kimmerle, G., and O.R.Klimmer. 1974. Acute and subchronic toxicity of sulfotep. Arch. Toxicol. 33:1–16.

Kimura, K.K., B.P.McNamara, and V.M.Sim. 1960. Intravenous administration of VX in man. U.S. Army Chemical Research and Development Laboratory, Technical Report No. 3017. Army Chemical Center, MD.

Klimmer, O.R., ed. 1971. Pflanzenschutz-und Schaedlingsbekaempfungsmittel: Abriss einer Toxikologie und Therapie von Vergiftungen, 2nd Ed. Hattingen, Fed. Rep. Germany.

Koelle, G.B. 1976. Cholinesterases and anticholinesterase agents. Berlin: Springer-Verlag. Pp. 123–154.

Koelle, G.B. 1981. Organophosphate poisoning—an overview. Fundam. Appl. Toxicol. 1:129–134.

Koelle, G.B. 1994. Pharmacology of organophosphates. J. Appl. Toxicol. 14: 105–109.

Koon, W.S., P.Cresthull, J.W.Crook, et al. 1959. Odor detection of VX vapor with and without a stabilizer. Chemical Warfare Laboratory Report, No. 2292. Aberdeen Proving Ground, MD.

Koon, W.S., J.W.Crook, and F.W.Oberst. 1960. Progress Report on the Toxicity of VX Vapor to Mice and Goats. Army Chemical Warfare Laboratories Report, No. TM 24–37. Army Chemical Center, MD.

Koplovitz, I., W.Harris, D.R.Anderson, W.J.Lennox, and J.R.Stewart. 1992. Reduction by pryidostigmine pretreatment of the efficacy if atropine and 2-PAM treatment of sarin and VX poisoning in rodents. Fund. Appl. Toxicol. 18:102–106.

Kotler-Cope, S., J.B.Milby, et al. 1996. Neuropsychological deficits in Persian Gulf War veterans: A preliminary report. Presented at the annual meeting of the International Neuropsychological Society, Chicago, IL.

Krackow, E.H. 1956. Toxicology of the V agents. CWLR 2065. Chemical Corps. Research and Development Command, Chemical Warfare Laboratories, Army Chemical Center , Aberdeen Proving Ground, MD.

Kujiraoka, T., T.Oka, M.Ishihara, T.Egashira, T.Fujioka, E.Saito, S.Saito, N. Miller, and H.Hattori. 2000. A sandwich enzyme-linked immunosorbent assay for human serum paraoxonase concentration. J. Lipid Research 41:1358–1363.

Laborde J.B., and H.K.Bates. 1986. Developmental toxicity study of agent GB-DCSM Types I and II in CD rats and NZW rabbits. Final Report. AD-A168 331. National Center for Toxicological Research, Jefferson, AR.

LaBorde, J.B., J.K.Bates, J.C.Dacre, and J.F.Young. 1996. Developmental toxicity of sarin in rats and rabbits. J. Toxicol. Environ. Health 47:249–265.

LaDu, B.N., J.I.Piko, H.W.Erickson, M.Vincent-Viry, and G.Seist. 1986. An improved method for phenotyping individuals for the human serum paraoxonase arylesterase polymorphism. Ann Biol. Clin. 44:369–372.

Lallement, G., P.Carpenteir, I.Pernot-Marino, D.Baubichon, A.Collet, and G. Blanchet. 1991 a. Involvement of the different rat hippocampal glutamatergic receptors in development of seizures induced by soman. Neurotoxicology 4:655–664.

Lallement, G., P.Carpenteir, A.Collet, I.Pernot-Marino, D.Baubichon, and G. Blanchet. 1991b. Effects of soman-induced seizures on different extracellular amino acid levels and on glutamate uptake in rat hippocampus. Brain Res. 563:234–340.

Landauer, M.R., and J.A.Romano. 1984. Acute behavioral toxicity of the organophosphate sarin in rats. Neurobehav. Toxicol. Teratol. 6:239–243.

Landrigan, P.J. 1997. Illness in Gulf war veterans, causes and consequences. JAMA 277:259–261.

Langenberg, J.P., E.T.Spruit, H.van der Wiel, et al. 1998. Inhalation toxicokinetics of soman stereoisomers in the atropinized guinea pig with nose-only exposure to soman vapor. Toxicol. Appl. Pharmacol. 151:79–87.

Lehmann, H., and J.Liddell. 1969. Human cholinesterase genetic variants and their recognition. Br. J. Anaesth. 41:235–244.

Lenz, D.E., D.M.Maxwell, and L.W.Austin. 1996. Development of a rat model for subacute exposure to the toxic organophosphate VX. J. Amer. College Toxicol. 15(suppl. 2):S69–S77.

Lessenger, J.E, and B.E.Reese. 1999. Rational use of cholinesterase activity testing in pesticide poisoning. J. Am. Board Fam. Pract. 12:307–314.

Lillibridge, S. 1995. Trip Report: United States Medical Delegation to Japan. From S.R.Lillibridge, Acting Chief, Disaster Assessment and Epidemiology Section, to R.J.Jackson, Director, National Center for Environmental Health, Centers for Disease Control and Prevention, Department of Health and Human Services, Atlanta, GA (25 April 1995).

Lipp, J., and T.Dola. 1980. Comparison of the efficacy of HS-6 versus HI-6 when combined with atropine, pyridostigmine and clonazepam for soman poisoning in the monkey. Arch. Int. Pharmacodyn. 246:138–148.

Little, J.S., C.A.Broomfield, M.K.Fox-Talbot, L.J.Boucher, B.MacIver, and D.E. Lenz. 1989. Partial characterization of an enzyme that hydrolyzes sarin, soman, tabun and diisopropyl phosphonofluoridate (DFP). Biochem. Pharmacol. 38:23–29.

Little, P.J., M.L.Reynolds, E.R.Bowman, and B.R.Martin. 1986. Tissue disposi

tion of ^{3}H sarin and its metabolites in mice. Toxicol. Appl. Pharmacol. 83:412– 419.

Little, P.J., J.A.Scimeca, and B.R.Martin. 1988. Distribution of [^{3}H] diisopropylfluorophosphate, [^{3}H] soman, [^{3}H] sarin and their metabolites in mouse brain. Drug Metab. Disposition 16:515–520.

Lohs, K, von. 1960. Zur Toxikologie und Pharmakologie organischer Phosphorsäureester. Dtsch Gesundheitsweren 15:2133–2179.

Lubash, G.D., and B.J.Clark. 1960. Some metabolic studies in humans following percutaneous exposure to VX. U.S. Army Chemical Research and Developmental Laboratories, Technical Report No. 3003. Edgewood Arsenal, MD.

Luo, C., and J.Liang. 1997. Evaluation of the combined toxic effects of GB/GF and efficacy of Jielin injection against combined poisoning in mice. Toxicol. Letters 92:195–200.

Maelicke, A., M.Samochocki, R.Jostock, A.Fehrenbacher, J.Ludwig, E.X.Albuquerque, and M.Zerlin. 2001. Allosteric sensitization of nicotinic receptors by galantamine, a new treatment strategy for Alzheimer's disease. Biol. Psychiatry 49:279–288.

Mager, P.P. 1981. Grouped observations in quantitative structure-toxicity relationships of organophosphorus poisons. Pharmazie 36:513–514.

Marrs, T.C., R.L.Maynard, and F.R.Sidell. 1996. Chemical Warfare Agents; Toxicology and Treatment. Chichester, NY: John Wiley and Sons.

Maxwell, D.M. 1992. The specificity of carboxylesterase protection against the toxicity of organophosphorus compounds. Toxicol. Appl. Pharmacol. 114:306–312.

Maxwell, D.M., K.M.Brecht, and B.M.O'Neill. 1987. The effect of carboxylesterase inhibition on interspecies differences in soman toxicity. Toxicol. Lett. 39:35–42.

McGrath, F.P., V.J.Von Berg, and F.W.Oberst. 1953. Toxicity and perception of GF vapor. Medical Laboratory Research Report, MLRR 185. Army Chemical Center, MD.

McKee, W.H.E., and B.Woolcott. 1949. Report on exposures of unprotected men and rabbits to low concentrations of nerve gas vapour. PRP-143. Chemical Defence Establishment, Porton Down, UK.

McNamara, B.P., and F.Leitnaker. 1971. Toxicological basis for controlling emission of GB into the environment. Edgewood Arsenal Special Publication 100–98 . Department of the Army, Edgewood Arsenal, Medical Research Laboratory, Edgewood Arsenal, MD.

McNamara, B.P., F.J.Vocci, and F.C.Leitnaker. 1973. Proposed limits for human exposure to VX vapor in nonmilitary operations, Edgewood Arsenal Special Publication, EASP 1100–1 (R-1). Aberdeen Proving Ground, MD.

McPhail, M.K., and P.A.Adie. 1960. The distribution of radioactive phosphorus in the blood and tissues of rabbits treated with tagged isopropyl methylphosphonofluoridate (Sarin) Can J. Biochem. Physiol. 38:945–951.

Mehl, A., T.M.Schenke, B.J.Johnsen, and F.Fonnum. 1994. The effects of tri

chlorfon and other organophosphates on prenatal brain development in the guinea pig. Neurochem. Res. 19:569–574.

Mioduszewski, R.J., S.A.Reutter, L.L.Miller, E.J.Olajos, and S.A.Thomson. 1998. Evaluation of airborne exposure limits for G-agents: Occupational and general population exposure criteria. ERDEC-TR-489, Edgewood Research Development and Engineering Center, Aberdeen Proving Ground, MD.

Mioduszewski, R.J., J.Manthei, R.Way, D.Burnett, B.Gaviola, W.Muse, S. Thomson, D.Sommerville, and R.Crosier. 2000. Estimating the probability of sarin vapor toxicity in rats as a function of exposure concentration and duration. Proceedings of the International Chemical Weapons Demilitarization Conference (CWD-2000), The Hague, NL. May 21–24, 2000.

Mioduszewski, R.J., J.Manthei, R.Way, D.Burnett, B.Gaviola, W.Muse, J. Anthony, D.Durst, D.Sommerville, R.Crosier, S.Thomson, and C.Crouse. 2001. ECBC Low Level Operational Toxicology Program: Phase I-Inhalation toxicity of sarin vapor in rats as a function of exposure concentration and duration. ECBC-TR-183, Edgewood Research Development and Engineering Center, Aberdeen Proving Ground, MD (August 2001).

Mioduszewski, R.J., J.Manthei, R.Way, D.Burnett, B.Gaviola, W.Muse, S. Thomson, D.Sommerville, and R.Crosier. 2002a. Interaction of exposure concentration and duration in determining acute toxic effects of sarin vapor in rats. Toxicol. Sci. 66:176–184.

Mioduszewski, R.J., J.Manthei, R.Way, D.Burnett, B.Gaviola, W.Muse, S. Thomson , D.Sommerville, R.Crosier, J.Scotto, D.McCaskey, C.Crous, and K.Matson. 2002b. Low-level sarin vapor exposure in rats: Effect of exposure concentration and duration on pupil size. ECBC-TR-235. Edgewood Chemical Biological Center, U.S. Army Soldier and Biological Chemical Command, Aberdeen Proving Ground, MD (May 2002).

MODa (Ministry of Defense). Unpublished report. Detection of G gases by smell. United Kingdom, Ministry of Defense. (As cited in Marrs et al. 1996.)

Morgan, D.P. 1989. Recognition and Management of Pesticide Poisonings. 4th Ed. EPA-540/9–88–001. U.S. Environmental Protection Agency, Washington, D.C.

Morita, H., N.Yanagisawa, T.Nakajima, M.Shimizu, H.Hirabayashi, H.Okudera, M.Nohara, Y.Midorikawa, and S.Mimura. 1995. Sarin poisoning in Matsumoto, Japan. The Lancet 346:290–293.

Mumford, S.A. 1950. A physiological assessment of the nerve gases. Porton Memorandum No. 39.

Munger, J.S., G.-P.Shi, E.A.Mark, D.T.Chin, C.Gerard, and H.A.Chapman. 1991. A serine esterase released by human alveolar macrophages is closely related to liver microsomal carboxylesterases. J. Biol. Chem. 266:18832– 18838.

Munro, N.B., A.P.Watson, K.R.Ambrose, and G.D.Griffin. 1990. Treating exposure to chemical warfare agents: Implications for health care providers and community emergency planning. Environ. Health Perspect. 89:205–215.

Munro, N.B., K.R.Ambrose, and A.P.Watson. 1994. Toxicity of the organophosphate chemical warfare agents GA, GB, and VX: Implications for public protection. Environ. Health Perspect. 102:18–38.

Munro, N.B., S.S.Talmage, S.G.D.Griffin, L.C.Waters, A.P.Watson, J.F.King, and V.Hauschild. 1999. The sources, fate, and toxicity of chemical warfare agent degradation products. Environ. Health Perspect. 107:933–974.

Murata, K., S.Araki, K.Yokoyama, T.Okumura, S.Ishimatsu, N.Takasu, and R.A.White. 1997. Asymptomatic sequelae to acute sarin poisoning in the central and autonomic nervous system 6 months after the Tokyo subway attack . J. Neurol. 244:601–606.

Nakajima, T., K.Sasaki, H.Ozawa, Y.Sekijima, H.Morita, Y.Fukushima, and N. Yanagisawa. 1998. Urinary metabolites of sarin in a patient of the Matsumoto sarin incident. Arch. Toxicol. 72:601–603.

NDRC (National Defense Research Committee). 1946. Chemical Warfare Agents and Related Chemical Problems. Part I–II, Vol. 1. National Defense Research Committee, Washington, DC.

Nieminen, S.A., A.Lecklin, O.Heikkinen, and P.Ylitalo. 1990. Acute behavioural effects of the organophosphates sarin and soman in rats. Pharmacol. Toxicol. 67:36–40.

NIOSH (National Institute for Occupational Safety and Health). 1999. Registry of Toxic Effects of Chemical Substances (RTECS) online file, retrieved November 1999. National Institute for Occupational Safety and Health, Cincinnati, OH. MEDLARS online information retrieval system, National Library of Medicine, Washington, DC.

Noort, D., A.G.Hulst, D.Platenburg, M.Polhuijs, and H.P.Benschop. 1998. Quantitative analysis of O-isopropyl methylphosphonic acid in serum samples of Japanese citizens allegedly exposed to sarin: Estimation of internal dose. Arch. Toxicol. 72:671–675.

Nordgren, I., G.Lundgren, G.Puu, and B.Holmstedt. 1984. Stereoselectivity of enzymes involved in toxicity and detoxification of soman. Arch. Toxicol. 55: 70–75.

NRC (National Research Council). 1985. Possible long-term health effects of short-term exposure to chemical agents. Vol. 3, Final Report: Current health status of test subjects. Committee on Toxicology, Board on Toxicology and Environmental Health Hazards, Commission on Life Sciences, National Research Council. Washington, DC: National Academy Press.

NRC (National Research Council). 1997. Review of the Acute Human-toxicity Estimates for Selected Chemical Warfare Agents. Committee on Toxicology, Subcommittee on Toxicity Values for Selected Nerve Agents and Vesicant Agents. Washington, DC: National Academy Press.

NRC (National Research Council). 1999. Review of the U.S. Army's Health Risk Assessment for oral exposure to six chemical warfare agents. Committee on Toxicology, Subcommittee on Chronic Reference Dose for Chemical Warfare Agents. Washington, DC: National Academy Press.

NRC (National Research Council). 2001. Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Chemicals. Committee on Toxicology, Subcommittee on Acute Exposure Guideline Levels. Washington, DC: National Academy Press.

Oberst, F.W. 1961. Factors affecting inhalation and retention of toxic vapors. Pp. 249–266 in Inhaled Particles and Vapours, C.N.Davis, ed. London: Pergamon Press.

Oberst, F.W., W.S.Koon, M.K.Christensen, et al. 1968. Retention of inhaled sarin vapor and its effect on red blood cell cholinesterase activity in man. Clin. Pharmacol. Ther. 9:421–427.

Okumura, T., N.Takasu, S.Ishimatu, S.Miyanoki, A.Mitsuhashi, K.Kumada, K. Tanaka, and S.Hinohara. 1996. Report on 640 victims of the Tokyo subway sarin attack. Ann. Emerg. Med. 28:129–135.

Olson, C.T., J.A.Blank, and R.G.Menton. 1998. Neuromuscular effects of low level exposures to sarin, pyridostigmine, DEET, and chlorpyrifos. Drug Chem. Toxicol. 21(Suppl. 1):149–169.

Olson, C.T., J.A.Blank, P.H.Kinney, A.W.Singer, G.B.Freeman, and R.A.Lordo. 2000. Neurologic assessment of rats following low doses of sarin, pyridostigmine, chlorpyrifos, and DEET. Presented at the 39th Annual Meeting of the Society of Toxicity, March, 2000, Philadelphia, PA. Toxicologist 54:265 (#1244).

Opresko, D.M., R.A.Young, R.A.Faust, S.S.Talmage, A.P.Watson, R.H.Ross, K.A.Davidson, and J.King. 1998. Chemical warfare agents: Estimating oral reference doses. Rev. Environ. Contam. Toxicol. 156:1–183.

Osweiler, G.D., T.L.Carson, W.B.Buck, and G.A.Van Gelder. 1985. Clinical and Diagnostic Veterinary Toxicology, 3rd Ed. Dubuque, IA: Kendall/Hunt.

OXO Process Panel. 1995. Letter report: Comments of the CMA OXO Process Panel on the Proposal to Reduce the TLV of N-Butyl Acetate. Written statement presented by the Chemical Manufacturers' Association OXO Process Panel to the ACGIH TLV Subcommittee. (Signed cover by G.D.Strickland, CMA Vice President, Technical Services, 25 Feb 1995, CMA, 2501 M. Street, NW, Washington, DC.)

Pearce, P.C., H.S.Crofts, N.G.Muggleton, D.Ridout, and E.A.M.Scott. 1999. The effects of acutely administered low dose sarin on cognitive behavior and the electroencephalogram in the common marmoset. J. Psychopharm. 13:128– 135.

Phillips, A. 1995. Clinical Toxicology Review, Massachusetts Poison Control Center. 17(7) [Online]. Available at http://www.maripoisoncenter.com/ctr/9504cholinesterase.html

Pond, A.L., H.W.Chambers, and J.E.Chambers. 1995. Organophosphate detoxication potential of various rat tissues via A-esterase and aliesterase activities. Toxicol. Letters 78:245–252.

Pope, C., and J.Liu. 2002. Nonesterase actions of anticholinesterase insecticides.

Chapter 3, pp. 29–43 in Handbook of Neurotoxicology, Vol. 1, E.J.Massaro, ed. Totowa, NJ: Human Press.

Pour-Jafari, H. 1994a. Congenital malformations in the progenies of Iranian chemical victims. Vet. Hum. Toxicol. 36:562–563.

Pour-Jafari, H. 1994b. Secondary sex ratios in progenies of Iranian chemical victims. Vet. Hum. Toxicol. 36:475–476.

Punte, C.L., and J.C.Atkinson. 1960. Inhalation toxicity and speed of action of VX with suggested estimates for man based on animal data. CWL Special Publication 2–30. U.S. Army Chemical Warfare Laboratories, Army Chemical Center, MD.

Rao, K.S., Y.Aracava, D.L.Rickett, and E.X.Albuquerque. 1987. Noncompetitive blockade of the nicotinic acetylcholine receptor-ion channel complex by an irreversible cholinesterase inhibitor. J. Pharmacol. Exp. Ther. 240:337–344.

Raveh, L., E.Grauer, J.Grunwald, E.Cohen, and Y.Ashani. 1997. The stoichiometry of protection against soman and VX toxicity in monkeys pretreated with human butyrylcholinesterase. Toxicol. Appl. Pharmacol. 145:43–53.

Reinhold, J.G., L.G.Tourigny, and V.L.Yonan. 1953. Measurement of serum cholinesterase activity by a photometric indicator method, together with a study of the influence of sex and race. Am. J. Clin. Pathol. 23:645–653.

Rengstorff, R.H. 1985. Accidental exposure to Sarin: Vision effects. Arch. Toxicol. 56:201–203.

Reutter, S.A., and J.V.Wade. 1994. "Table 1; Summary of Existing and Recommended Estimates (U)," unclassified summary table, Review of existing toxicity data and human estimates for selected chemical agents and recommended human toxicity estimates appropriate for defending the soldier. ERDEC-SP-018. U.S. Department of the Army, Edgewood Research Development and Engineering Center, Aberdeen Proving Ground, MD (secret report).

Reutter, S.A., R.J.Mioduszewski, and S.A.Thomson, S.A. 2000. Evaluation of airborne exposure limits for VX: Worker and general population exposure criteria. ECBC-TR-074. Edgewood Chemical Biological Center, U.S. Army Soldier and Biological Chemical Command, Aberdeen Proving Ground, MD.

Rider, J.A., J.L.Hodges, J.Swader, and A.D.Wiggins. 1957. Plasma and red cell cholinesterase in 800 "healthy" blood donors. J. Lab. Clin. Med. 50:376–383.

Rocha, E.S., S.R.Chebabo, M.D.Santos, Y.Aracava and E.X.Albuquerque. 1998. An analysis of low level doses of cholinesterase inhibitors in cultured neurons and hippocampal slices of rats. Drug Chem. Toxicol. 21(Suppl. 1):191–200.

Rocha, E.S., M.D.Santos, S.R.Chebabo, Y.Aracava and E.X.Albuquerque. 1999. Low concentrations of the organophosphate VX affect spontaneous and evoked transmitter release from hippocampal neurons: Toxicological relevance of cholinesterase-dependent actions. Toxicol. Appl. Pharmacol. 159:31–40.

Rubin, L.S., S.Krop, and M.N.Goldberg,. 1957. Effect of sarin on dark adaptation in man: Mechanism of action. J. Appl. Physiol. 11:445–449.

Rubin, L.S., and M.N.Goldberg. 1957. Effect of sarin on dark adaptation in man: Threshold changes. J. Appl. Physiol. 11:439–444.

Ruiz, J., H.Blanche, R.W.James, M.C.Garin, C.Vaisse, G.Charpentier, N.Cohen, A.Morabia, P.Passa, and P.Froguel. 1995. Gln-Arg 192 polymorphism of paraoxonase and coronary artery disease in type 2 diabetes. Lancet 346: 869–872.

Schoene, K., D.Hochrainer, H.Oldiges, M.Krügel, N.Franzes, and H.J.Bruckert. 1985. The protective effect of oxime pretreatment upon the inhalative toxicity of sarin and soman in rats. Fund. Appl. Toxicol. 5:S84–S88.

Schreider, J.P., J.R.Rowland, L.S.Rosenblatt, and A.G.Hendrick. 1984. The teratology effects of VX in rats. Draft Final Report. Laboratory for Energy-Related Health Effects Research. University of California, Davis, CA. Prepared for U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Derrick, MD.

Schwartz, D.A., B.N.Doebbelin, and J.A.Merchant. 1997. Self-reported illness and health status among Gulf War veterans. JAMA 277:238–245.

Senanayake, N., and L.Karalliedde. 1987. Neurotoxic effects of organophosphate insecticides: An Intermediate Syndrome. N. Engl. J. Med. 316:761–763.

Serrato, M., and M.J.Marian. 1995. A variant of human paraoxonase/arylesterase (HUMPONA) gene is a risk factor for coronary artery disease. J. Clin. Invest. 96:3005–3008.

Shanor, S.P., G.R.van Hees, N.Baart, E.E.Erdos, and F.F.Foldes. 1961. The influence of age and sex on human plasma and red cell cholinesterase. Am. J. Med. Sci. 242:357–361.

Shih, M.L., J.D.McMonagle, and T.W.Dolzine. 1994. Metabolite pharmacokinetics of soman, sarin and GF in rats and biological monitoring of exposure to toxic organophosphorus agents. J. Appl. Toxicol. 14:195–199.

Sidell, F.R. 1974. Soman and Sarin: Clinical manifestations and treatment of accidental poisoning by organophosphates. Clin. Toxicol. 7:1–17.

Sidell, F.R. 1990. What to do in case of an unthinkable: Chemical warfare attack or incident. Postgraduate Medicine 88:70–83.

Sidell, F.R. 1992. Clinical considerations in nerve agent intoxication. Pp. 155–194 in: Chemical Warfare Agents, S.M.Somani, ed. New York: Academic Press, Inc.

Sidell, F.R. 1996. Chemical agent terrorism. Ann. Emerg. Med. 28:223–224.

Sidell, F.R. 1997. Nerve agents. Pp. 129–179 in Medical Aspects of Chemical and Biological Warfare, F.R.Sidell, E.T.Takafuji, and D.R.Franz, eds. Published by the Office of the Surgeon General, at TBMM Publications, Borden Institute, Walter Reed Army Medical Center, Washington, DC.

Sidell, F.R. and W.A.Groff. 1974. The reactivatibility of cholinesterase inhibited by VX and sarin in man. Toxicol. Appl. Pharmacol. 27:241–252.

Sim,V.M. 1956. Effect on pupil size of exposure to GB vapour. Porton Technical Paper 531. Chemical Defence Experimental Establishment, Directorate of Chemical Defence Research and Development, Porton Down, England.

Sim, V.M. 1962. Variability of different intact human-skin sites to the penetration of VX. CRDLR 3122, Chemical Research and Development Laboratories, Aberdeen Proving Ground, MD.

Sim, V.M., C.McClure, Jr., F.J.Vocci, et al. 1964. Tolerance of man to VX-contaminated water. Technical Report CRDLR 3231. U.S. Department of the Army , Chemical Research and Development Laboratories, Edgewood Arsenal, MD.

Small, M.J. 1984. Compounds formed from the chemical decontamination of HD, GB, and VX and their environmental fate. Technical Report 8304 (DTIC: AD A149515). U.S. Department of the Army, Medical Bioengineering Research and Development Laboratory, Fort Derrick, MD.

Somani, S.M., R.P.Solana, and S.N.Dube. 1992. Toxicodynamics of nerve agents. Pp 67–123 in Chemical Warfare Agents, S.M.Somani, ed. New York: Academic Press, Inc.

Spruit, H.E., J.B.Langenberg, H.C.Trap, H.J.van der Wiel, R.B.Helmich, H.P. van Helden, and H.P.Benschop. 2000. Intravenous and inhalation toxicokinetics of sarin stereoisomers in atropinized guinea pigs. Toxicol. Appl. Pharmacol. 169(3):249–254.

Sterri, S.H., S.Lyngaas, and F.Fonnum. 1980. Toxicity of soman after repetitive injection of sublethal doses in rat. Acta Pharmacol. Toxicol. 46:1–7.

Sterri, S.H., O.Kloster, and G.Valdal. 1982. Reduction of blood cholinesterase activities following administration of soman by different routes in guinea pigs. Acta Pharmacol. Toxicol. 50:326–331.

Storm, J.E., K.K.Rozman, and J.Doull. 2000. Occupational exposure limits for 30 organophosphate pesticides based on inhibition of red blood cell cholinesterase. Toxicology 150:1–29.

ten Berge, W.F., A.Zwart, and L.M.Appelman. 1986. Concentration-time mortality response relationship of irritant and systemically acting vapours and gases. J. Hazard. Materials 13:301–309.

Thacker, S.B., Assistant Surgeon General, Acting Director, National Center for Environmental Health, Center for Disease Control and Protection, U.S. Department of Health and Human Services. 1994. Letter establishing "Recommended Acute Threshold Effects Levels for Determining Emergency Evacuation Distances in the CSEPP Program," to Col. J.M.Coverstone, Deputy for Chemical Demilitarization, Office of the Assistant Secretary (I, L &E), The Pentagon, Washington, DC (24 June 1994).

Uhde, G.I., and R.F.Moore,. 1945. Eye effects of T2104. Porton Technical Report 2693. CDE Porton Down, England.

USACHPPM (U.S. Department of the Army, Center for Health Promotion and Preventive Medicine). 1996. Detailed and general facts about chemical agents. Technical Guide 218 (TG-218). U.S. Army Center for Health Promotion and Preventive Medicine, 5158 Blackhawk Road, Aberdeen Proving Ground, MD.

USACHPPM (U.S. Army Center for Health Promotion and Preventive Medicine). 1998. Memorandum for Record (12 November 1998) prepared by V. Hauschild, Environmental Health Risk Assessment and Risk Communication Program. In Progress Review: Meeting Regarding Evaluation of Airborne Exposure Limits for VX, held Oct. 14, 1998, Aberdeen Proving Ground, MD.

USACMDA (U.S. Department of the Army, Chemical Materiel Destruction Agency). 1993a. Interim survey and analysis report. Program Manager for Non-Stockpile Chemical Materiel, Non-Stockpile Chemical Materiel Program, U.S. Department of the Army, Chemical Materiel Destruction Agency, Aberdeen Proving Ground, MD.

USACMDA (U.S. Department of the Army, Chemical Materiel Destruction Agency). 1993b. Survey and analysis report. Program Manager for Non-Stockpile Chemical Materiel Program, U.S. Department of the Army, Chemical Materiel Destruction Agency, Aberdeen Proving Ground, MD.

van Helden, H.P.M., J.P.Langenberg, and H.P.Benschop. 2001. Low Level Exposure to GB Vapor in Air: Diagnosis/Dosimetry, Lowest Observable Effect Levels, and Performance Incapacitation. Award No. DAMD17–97–1–7360. TNO Prins Maurits Laboratory, Final Report to the U.S. Army Medical Research and Materiel Command, Fort Derrick, MD (April 2001).

van Helden, H.P.M. H.C.Trap, W.C.Kuijpers, B.Groen, J.P.Oostdijk, R.A.P. Vanwersch, I.H.C.Philippens, J.P.Langenberg, and H.P.Benschop. 2002. Low Level Exposure to GB Vapor in Air: Diagnosis/Dosimetry, Lowest Observable Effect Level, and Performance-Incapacitation. Research and Technology Organisation (RTO) Meeting Proceedings 75, Operational Medical Issues in Chemical and Biological Defense [RTO-MP-075, AC/323 (HFM-060) TP/37] held in Estoril, Portugal, 14–17 May 2001. North Atlantic Treaty Organisation, Research and Technology Organisation, BP 25, 7 Rue Ancelle, F-92201 Neuilly-sur-Seine CEDEX , France.

Van Kampen, K.R., Shupe, J.L., Johnson, A.E., et al. 1970. Effects of nerve gas poisoning in sheep in Skull Valley, Utah. J. Amer. Vet. Med. Assoc. 156: 1032–1035.

Vasterling, J.J., K.Brailey, J.I.Constans, and P.B.Sutker. 1998. Attention and memory dysfunction in posttraumatic stress disorder. Neuropsychology 12:125–133.

Vranken, M.A., H.C.DeBisschop, and J.L.Willems. 1982. In vitro inhibition of neurotoxic esterase by organophosphorous nerve agents. Arch. Int. Pharmacodyn. Ther. 260:316–318.

Wahllander, A., and L.Szinicz. 1990. Detoxification of soman in the perfused rat liver: Quantitative uptake and stereoisomer metabolism. Arch. Toxicol. 64: 586–589.

Walday, P., P.Aas, and F.Fonnum. 1991. Inhibition of serine esterases in different rat tissues following inhalation of soman. Biochem. Pharmacol. 41(1):151– 153.

Wang, Q., M.Sun, H.Zhang, and C.Huang. 1998. Purification and properties of soman-hydrolyzing enzyme from human liver. J. Biochem. Molecular Toxicol. 12(4):213–217.

Watson, A.P., K.R.Ambrose, G.D.Griffin, S.S.Leffingwell, N.B.Munro, and L.C. Waters,. 1989. Health effects of warfare agent exposure: Implications for stockpile disposal. Environ. Prof. 11:335–353.

Watson, A.P., T.D.Jones, and J.D.Adams. 1992. Relative potency estimates of acceptable residues and reentry intervals after nerve agent release. Ecotoxicology and Environ. Safety 23:328–342.

Weimer, J.T., B.P.McNamara, E.J.Owens, J.G.Cooper, and A.van de Wal. 1979. Proposed revision of limits for human exposure to GB vapor in nonmilitary operations based on one-year exposures of laboratory animals to low airborne concentrations. ARCSL-TR-78056. U.S. Army Armament Research and Development Command, Chemical Systems Laboratory, Aberdeen Proving Ground, MD.

Wells, C.H., M.T.Collins, D.E.Vanderveer, and W.A.Wells. 1993. Standards for chemical and biological warfare hazard modeling, Volume I. Toxicity of nerve agents and mustard in humans. Abridged edition (unclassified). AL-TR-1993– 0026, Jaycor, Beavercreek, OH. Report to the U.S. Air Force, Armstrong Laboratory, Ergonomics Analysis Branch, Wright-Patterson Air Force Base, OH .

Willems, J.L., M.Narcaise, and H.C.DeBisschop. 1984. Delayed neuropathy by the organophosphorous nerve agents soman and tabun. Arch. Toxicol. 55:76– 77.

Wills, J.H. 1972. The measurement and significance of changes in the cholinesterase activities of erythrocytes and plasma in man and animals. CRC Crit. Rev. Toxicol. 1:153–202.

Wilson, B.W., J.D.Henderson, T.P.Kellner, M.Goldman, R.J.Higgins, and J.C. Dacre. 1988. Toxicity of repeated doses of organophosphorous esters in the chicken. J. Toxicol. Environ. Health 23:115–126.

Wilson, B.W., T.G.Kawakami, N.Cone, J.D.Henderson, L.S.Rosenblatt, and M. Goldman. 1994. Genotoxicity of the phosphoramidate agent tabun (GA). Toxicology 86:1–12.

Windholz, M., S.Budavari, R.F.Blumetti, and E.S.Otterbein, eds 1983. The Merck Index, An Encyclopedia of Chemicals, Drugs, and Biologicals. 10th Ed. Rahway, NJ: Merck and Co.

Wolthuis, O.L. 1992. Behavioral effects of low doses of cholinesterase inhibitors in robot-tested marmosets. DTIC: AD A262776. Prepared for the U.S. Department of the Army, Medical Research and Development Command, Fort Detrick, MD. TNO Medical Biological Laboratory, Rijswijk, Netherlands.

Woodard, C.L., C.A.Calamaio, A.Kaminskis, D.A.Anderson, L.W.Harris, and D.G.Martin. 1994. Erythrocyte and plasma cholinesterase activity in male and female rhesus monkeys before and after exposure to sarin. Fund. Appl. Toxicol. 23:342–347.

Yamasaki, Y., K.Sakamoto, H.Watada, Y.Kajimoto, and M.Hori. 1997. The Arg 192 isoform of paraoxonase with low sarin-hyrolyzing activity is dominant in the Japanese. Japan. Hum. Genet. 10:67–68.

Yee, E., J.Armour, and R.Bide. 1999. An approach to obtain estimates of human toxicity. Part II: A three dimensional probit based, nonlinear dose response model for calculation of the mortality-concentration-time response surface. Presented to the NATO Challenge Subgroup, 12–14 May, 1999, San Antonio, TX.

Yee, E. 1996. A Non-linear Dose-Response Model with an Application to the Reconstruction of the Human Mortality Response Surface from Acute Inhalation Toxicity with Sarin. DRES-SM-1476. Defence Research Establishment Suffield, Ralston, Alberta, Canada.

Yokoyama, K., S.Araki, K.Murata, M.Nishikitani, T.Okumura, S.Ishimatsu, and N.Takasu. 1998a. Chronic neurobehavioral effects of Tokyo subway Sarin poisoning in relation to posttraumatic stress disorder. Arch. Environ. Health 53:249–256.

Yokoyama, K., S.Araki, K.Murata, M.Nishikitani, T.Okumura, S.Ishimatsu, N. Takasu, and R.A.White. 1998b. Chronic neurobehavioral and central and autonomic nervous system effects of Tokyo subway Sarin poisoning. J. Physiol. (Paris) 92:317–323.

Yokoyama, K., S.Araki, K.Murata, M.Nishikitani, T.Okumura, S.Ishimatsu, and N.Takasu. 1998c. A preliminary study of vestibulocerebellar effects of Tokyo subway sarin poisoning in relation to gender differences: Frequency analysis of postural sway. J. Occup. Environ. Med. 40:17–21.

Young, R.A., D.M.Opresko, A.P.Watson, R.H.Ross, J.King, and H.Choudhury. 1999. Deriving toxicity values for organophosphate nerve agents: A position paper in support of the procedures and rationale for deriving oral RfDs for chemical warfare nerve agents. Human Ecol. Risk Assess. 5:589–634.

Young, J.F., B.J.Gough, R.L.Suber, and D.W.Gaylor. 2001. Correlation of blood cholinesterase levels with toxicity of sarin in rats. J. Toxicol. Environ. Health 62:161–174.

Zhao, D.-L., Z.-X. Wang, S.-Q. Pei, and C.-H.Liu. 1983. Effects of soman, sarin, and VX on the specific binding of 3H-QNB in rat cerebral cortex homogenates. Acta. Pharmacol. Sinica 4:225–228.

Appendixes

APPENDIX A

DERIVATION OF AEGL VALUES

Derivation of AEGL-1

Key study:	Mioduszewski et al. (2002b). Miosis measured in adult female SD rats exposed to a range of GB vapor concentrations for 10, 60, and 240 min. At 10 min, *N* =52 females; at 60 min, *N*=35 females; at 240 min, *N*=55 females.
Toxicity end point:	EC_{50} for miosis (defined as "a postexposure pupil diameter 50% or less of the preexposure pupil diameter") observed in adult female SD rats
EC_{50} for GB:	10-min EC_{50}=0.068 mg GB/m3 60 min EC_{50}=0.020 mg GB/m3 240 min EC_{50}=0.012 mg GB/m3
Scaling:	$C^n \times t = k$ (ten Berge et al. 1986). Current analyses based on a linear regression of the lethality and miosis data for female SD rats. The LC_{01} of GB to female Sprague-Dawley rats (Mioduszewski et al. 2000, 2001, 2002a) yields an *n* value of 1.93 with an r^2 of 0.9948, while miosis (female Sprague-Dawley rats; Mioduszewski et. al. 2002b) yields an *n* value of 2.00 with an r^2 of 0.4335 (see Appendix B). The anticholinesterase mechanism of mammalian toxicity for nerve agents is known, and all endpoints observed in human and animal studies represent a response continuum to anticholinesterase exposure. Accordingly, it is valid to apply an n value derived from compound-specific lethality data to time scaling for non-lethal effects, and an n value derived for miosis, to consideration of other toxic effects resulting from anticholinesterase exposure. This position is consistent with that of the recently published Science

	Policy of the EPA Office of Pesticide Programs (EPA 2000). Therefore, n=2 is used as the scaling function for all AEGL time point derivations.
Uncertainty factors:	Interspecies: 1 (miosis response to GB vapor is similar across mammal species) Intraspecies: 10 (adjustment for possible susceptible individuals)
Calculations:	(C/uncertainty factors)^2×t=k 10-min to 30-min extrapolation ([0.068 mg/m^3]/10)^2×(10/60) h=k 7.7×10^{-6} mg/m^3 h=k 4-h to 8-h extrapolation ([0.012 mg/m^3]/10)^2×4 h=k 5.8×10^{-6} mg/m^3 h=k
10-min AEGL-1:	C=0.068 mg/m^3 10-min AEGL-1=(0.068 mg/m^3)/10=0.0068 mg/m^3
30-min AEGL-1:	C^2×(0.5 h)=(7.7×10^{-6} mg/m^3)×h 30-min AEGL-1=0.0039 mg/m^3
1-hAEGL-1:	C=0.020 mg/m^3 1-h AEGL-1=(0.020 mg/m^3)/10=0.0020 mg/m^3
4-h AEGL-1:	C=0.012 mg/m^3 4-h AEGL-1=(0.012 mg/m^3)/10=0.0012 mg/m^3
8-h AEGL-1:	C^2×(8 h)=(5.8×10^{-6} mg/m^3)×h 8-h AEGL-1=0.00085 mg/m^3=0.001 mg/m^3

Derivation of AEGL-1 for Agent GB (Sarin) from Human Data Set

Key study:	Harvey (1952) (see also Johns [1952])

Toxicity end point:	Rhinorrhea, headache, tightness in chest, cramps, nausea, and miosis (mean maximal decrease in pupil diameter) observed in human volunteers exposed to GB at 0.05 mg/m^3 for 20 min. Multiple observations at GB vapor Cts ranging from 0.0 to 6.0 mg·min/m^3.
LOAEL for GB:	0.05 mg/m^3 for 20 min
Scaling:	$C^n \times t = k$ (ten Berge et al. 1986). Current analyses based on same logic as for AEGL-1 derivation from female SD rat miosis data of Mioduszewski et al (2002b).
Uncertainty factors:	Interspecies: 1 (human data were used) Intraspecies: 10 (adjustment for possible susceptible individuals)
Calculations:	$(C/\text{uncertainty factors})^2 \times t = k$ $([0.05\ \text{mg/m}^3]/10)^2 \times (20/60)\ \text{h} = k$ $8.25 \times 10^{-6}\ \text{mg/m}^3 \times \text{h} = k$
10-min AEGL-1:	$C^2 \times (0.167\ \text{h}) = (8.25 \times 10^{-6}\ \text{mg/m}^3) \times \text{h}$ $C = 0.0069\ \text{mg/m}^3$
30-min AEGL-1:	$C^2 \times (0.5\ \text{h}) = (8.25 \times 10^{-6}\ \text{mg/m}^3) \times \text{h}$ $C = 0.0040\ \text{mg/m}^3$
1-h AEGL-1:	$C^2 \times (1\ \text{h}) = (8.25 \times 10^{-6}\ \text{mg/m}^3) \times \text{h}$ $C = 0.0028\ \text{mg/m}^3$
4-h AEGL-1:	$C^2 \times (4\ \text{h}) = (8.25 \times 10^{-6}\ \text{mg/m}^3) \times \text{h}$ $C = 0.0014\ \text{mg/m}^3$
8-h AEGL-1:	$C^2 \times (8\ \text{h}) = (8.25 \times 10^{-6}\ \text{mg/m}^3) \times \text{h}$ $C = 0.0010\ \text{mg/m}^3$

Derivation of AEGL-2 for Agent GB (Sarin)

Key study:	Baker and Sedgwick (1996). Eight healthy male servicemen exposed to GB at 0.5 mg/m^3 for 30 min in an exposure chamber. During the exposure, test subjects walked at a rate of 96 paces per minute and breathed normally.
Toxicity end point:	Observed effects include miosis in eight of eight subjects, dyspnea and photophobia in some individuals (number not given), inhibition of RBC-ChE to approximately 60% (range of 54–66%) of individual baseline at 3 h and 3 d postexposure in eight of eight subjects, and small but measurable changes in single fibre electromyography (SFEMG) of the forearm that were detectable in the lab between 4 and 15 mo postexposure (five of eight subjects). Respiratory effects resolved within minutes; ocular effects resolved within 48 h. Electromyographic changes considered subclinical by study authors. No clinical effects.
GB concentration:	0.5 mg/m^3 for 30 min
Scaling:	$C^n \times t = k$ (ten Berge et al. 1986); n=2, see earlier discussion of AEGL-1 scaling
Uncertainty factors:	Interspecies: 1 (human data were used) Intraspecies: 10 (for possible susceptible individuals)
Calculations:	(C/uncertainty factors)^2×t=k
	([0.5mg/m^3]/10)^2×0.5 h=k 0.0013 mg/m^3×h=k
10-min AEGL-2:	C^2×(0.167 h)=(0.0013 mg/m^3)×h C=0.087 mg/m^3
30-min AEGL-2:	C^2×(0.5 h)=(0.0013 mg/m^3)×h

30-min AEGL-2:	$C^2 \times (0.5\ h) = (0.0013\ mg/m^3) \times h$ $C = 0.050\ mg/m^3$
1-h AEGL-2:	$C^2 \times (1\ h) = (0.0013\ mg/m^3) \times h$ $C = 0.035\ mg/m^3$
4-h AEGL-2:	$C^2 \times (4\ h) = (0.0013\ mg/m^3) \times h$ $C = 0.017\ mg/m^3$
8-h AEGL-2:	$C^2 \times (8\ h) = (0.0013\ mg/m^3) \times h$ $C = 0.0125\ mg/m^3$

Derivation of AEGL-3 for Agent GB (Sarin)

Key study:	Mioduszewski et al. (2000, 2001, 2002a). Fourteen-day acute lethal toxicity of GB to female Sprague-Dawley rats was evaluated for time periods of 10, 30, 60, 90, 240, and 360 min in a whole-body dynamic chamber. Ten females were used for each concentration-time (Ct) combination, and 50 females were used for each time point. GB concentrations ranged from about 2 to 54 mg/m^3.
Toxicity end point:	Fourteen-day acute lethal toxicity of GB to female Sprague-Dawley rats. Female rats were reported to be more sensitive to GB vapor toxicity than males over the range of exposure concentrations and durations studied. Gender differences for lethality are reported by Mioduszewski et al. (2000, 2001, 2002a) to be statistically significant at $p<0.01$. Probit analysis (MINITAB, version 13) presented in Mioduszewski et al. (2000) gave the following 14-d LC_{50} and LC_{01} values for female rats: LC_{50} 18.1 mg/m^3 for 10 min in female rats 8.51 mg/m^3 for 30 min in female rats

	6.39 mg/m^3 for 60 min in female rats 3.03 mg/m^3 for 4 h in female rats 2.63 mg/m^3 for 6 h in female rats LC_{01} 11.54 mg/m^3 for 10 min in female rats 5.84 mg/m^3 for 30 min in female rats 4.01 mg/m^3 for 60 min in female rats 2.09 mg/m^3 for 4 h in female rats 1.76 mg/m^3 for 6 h in female rats
Scaling:	$C^n \times t = k$ *(ten* Berge et al. 1986); n=2, see earlier discussion of AEGL-1 scaling; to extrapolate from 6-h value to an 8-h estimate. Scaling to derive 8-h LC_{01}.
Uncertainty factors:	Interspecies: 3 (female rat data); full default value of 10 not considered appropriate since the mechanism of toxicity in rats and humans is the same, cholinesterase inhibition. Intraspecies: 10 (for possible susceptible individuals)
Calculations:	(C/uncertainty factors)$^2 \times t = k$ ([1.76mg/m^3]/30)$^2 \times$6.0 h=k 0.021mg/m$^3 \times$h=k
10-min AEGL-3:	C=11.54 mg/m^3 10-min AEGL-3=(11.54 mg/m^3)/30=0.38 mg/m^3
30-min AEGL-3:	C=5.84 mg/m^3 30-min AEGL-3=(5.84 mg/m^3)/30=0.194 mg/m^3
1-h AEGL-3:	C=4.01 mg/m^3 1-h AEGL-3=(4.01 mg/m^3)/30=0.133 mg/m^3
4-h AEGL-3:	C=2.09 mg/m^3 4-h AEGL-3=(2.09 mg/m^3)/30=0.069=0.070 mg/m^3

8-h AEGL-3:	$C^2 \times (8\ h) = (0.021\ mg/m^3) \times h$ 8-h AEGL-3=0.051 mg/m^3

Derivation of AEGL-3 for Agent GD (Alternate Estimate)

Key study:	Aas et al. (1985)
Toxicity end point:	Threshold for mortality in rats estimated to be 335 mg·min/m^3 for an exposure period of 16 min. The lethal threshold is equivalent to an exposure to a GD concentration of 21 mg/m^3 for 16 min.
Scaling:	Data insufficient to derive an *n* value from the Aas et al. (1985) study. The anticholinesterase mechanism of mammalian toxicity for nerve agents is known, and all end points observed in human and animal studies represent a response continuum to anticholinesterase exposure. Accordingly, it is valid to apply an *n* value derived from the compound-specific lethality and miosis data for nerve agent GB (Mioduszewski et al. 2000, 2002, 2002a,b) to time scaling for all AEGL effects. This position is consistent with that of the recently published science policy of the EPA Office of Pesticide Programs (EPA 2000). Therefore, $n=2$ is used as the scaling function for all AEGL time point derivations for the G-series nerve agents.
Uncertainty factors:	Interspecies: 10 (sparse data set for agent GD) Intraspecies: 10 (for possible susceptible individuals; sparse data set for agent GD)
Calculations:	$(C/\text{uncertainty factors})^2 \times t = k$ $([21\ mg/m^3]/100)^2 \times (16/60)\ h = 0.012\ mg/m^3 \times h$
10-min AEGL-3:	$C^2 \times (0.167) = (0.012\ mg/m^3) \times h$

30-min AEGL-3:	10-min AEGL-3=0.27 mg/m^3 $C^2\times(0.5\text{ h})=(0.012\text{ mg/m}^3)\times\text{h}$ 30-min AEGL-3=0.15 mg/m^3

Derivation of AEGL-1 for Agent VX Vapor from Agent GB Toxicity Data

Key study:	Mioduszewski et al. (2002b). Miosis measured in adult female SD rats exposed to a range of GB vapor concentrations for 10, 60, and 240 min. At 10 min, N =52 females; at 60 min, N=35 females; at 240 min, N=55 females.
Toxicity end point:	EC_{50} for miosis (defined as "a postexposure pupil diameter 50% or less of the preexposure pupil diameter") observed in adult female SD rats
EC_{50} for GB:	10-min EC_{50}=0.068 mg/m^3 60-min EC_{50}=0.020 mg/m^3 240-min EC_{50}=0.012 mg/m^3
Estimated EC_{50} for VX:	10-min EC_{50}=0.017 mg/m^3 (based on relative potency of 4 as described in Section 4.3) 60-min EC_{50}=0.005 mg/m^3 (based on relative potency of 4 as described in Section 4.3) 240-min EC_{50}=0.003 mg/m^3 (based on relative potency of 4 as described in Section 4.3)
Scaling:	$C^n\times t$=k (ten Berge et al. 1986). Current analyses based on a linear regression of the lethality and miosis data for female SD rats. The LC_{01} of GB to female Sprague-Dawley rats (Mioduszewski et al. 2000, 2001, 2002a) yields an n value of 1.93 with an r^2 of 0.9948, while miosis (female Sprague-Dawley rats) (Mioduszewski et. al. 2002b) yields an n value of 2.00 with an r^2 of 0.4335. The anticholinesterase

	mechanism of mammalian toxicity for nerve agents is known, and all end points observed in human and animal studies represent a response continuum to anticholinesterase exposure. Accordingly, it is valid to apply an *n* value derived from compound-specific lethality data to time scaling for nonlethal effects, and an *n* value derived for miosis to consideration of other toxic effects resulting from anticholinesterase exposure. This position is consistent with that of the recently published science policy of the EPA Office of Pesticide Programs (EPA 2000). Therefore, $n=2$ is used as the scaling function for all AEGL time point derivations.
Uncertainty factors:	Interspecies: 1 (miosis response to GB vapor similar across mammal species) Intraspecies: 10 (adjustment for possible susceptible individuals)
Modifying factor:	3 (for sparse VX data base)
Calculations:	$(C/\text{uncertainty factors})^2 \times t = k$
	10-min to 30-min extrapolation $([0.017\ mg/m^3]/30)^2 \times (10/60)\ h = k$ $5.0 \times 10^{-8}\ mg/m^3 \times h = k$ 4-h to 8-h extrapolation $([0.003\ mg/m^3]/30)^2 \times 4\ h = k$ $4.0 \times 10^{-8}\ mg/m^3 \times h = k$
10-min AEGL-1:	$C = 0.017\ mg/m^3$ 10-min AEGL-1$=(0.017\ mg/m^3)/30=0.00057\ mg/m^3$
30-min AEGL-1:	$C^2 \times (0.5\ h) = (5.0 \times 10^{-8}\ mg/m^3) \times h$ 30-min AEGL-1$=0.00033\ mg/m^3$
1-h AEGL-1:	$C = 0.0050\ mg/m^3$

4-hAEGL-1:	C=0.003 mg/m^3 4-h AEGL-1=(0.003 mg/m^3)/30=0.00010 mg/m^3
8-hAEGL-1:	$C^2\times(8\text{ h})=(4.0\times10^{-8}\text{ mg/m}^3)\times\text{h}$ 8-h AEGL-1=0.000071 mg/m^3

Derivation of AEGL-2 for Agent VX Vapor from Agent GB Toxicity Data

Key Study:	Baker and Sedgwick (1996)
Toxicity end point:	Miosis in all subjects, dyspnea and photophobia in some individuals, inhibition of RBC-ChE to approximately 60% of individual baseline at 3 h and 3 d postexposure, and small but measurable changes in single fibre electromyography (SFEMG) of the forearm that were detectable in the lab between 4 and 15 mo postexposure. Baker and Sedgwick (1996) considered these SFEMG changes to be subclinical and reversible. Current analysis considered SFEMG changes to be a protective interpretation of the AEGL-2 definition.
GB concentration:	0.5 mg/m^3 for 30 min
Estimated VX concentration:	0.125 mg/m^3 for 30 min (based on relative potency of 4 for miosis and mild effects as described in Section 4.3)
Scaling:	$C^n\times t=k$ (ten Berge et al. 1986). The n value of 2 used for agent GB is also used for agent VX (see text)
Uncertainty factors:	Interspecies: 1 (human data were used) Intraspecies: 10 (adjustment for possible susceptible individuals)

	individuals)
Modifying factor (MF):	3 (for sparse VX data base)
Calculations:	$(C/[\text{uncertainty factors}\times\text{MF}])^2\times t=k$
	$([0.125\ \text{mg/m}^3]/[10\times3])^2\times0.5\ \text{h}= 8.7\times10^{-6}\ \text{mg/m}^3\times\text{h}$
10-min AEGL-2:	$([8.7\times10^{-6}\ \text{mg/m}^3]/0.1667\ \text{h})^{1/2}=0.0072\ \text{mg/m}^3$
30-min AEGL-2:	$([8.7\times10^{-6}\ \text{mg/m}^3]/0.5\ \text{h})^{1/2}=0.0042\ \text{mg/m}^3$
1-h AEGL-2:	$([8.7\times10^{-6}\ \text{mg/m}^3]/1\ \text{h})^{1/2}=0.0029\ \text{mg/m}^3$
4-h AEGL-2:	$([8.7\times10^{-6}\ \text{mg/m}^3]/4\ \text{h})^{1/2}=0.0015\ \text{mg/m}^3$
8-h AEGL-2:	$([8.7\times10^{-6}\ \text{mg/m}^3]/8\ \text{h})^{1/2}=0.00104\ \text{mg/m}^3$

Derivation of AEGL-3 for Agent VX Vapor from Agent GB Toxicity Data

Key study:	Mioduszewski et al. (2000, 2001, 2002a)
Toxicity end point:	GB-induced lethality in female Sprague-Dawley rats
GB LC_{50}:	18.1 mg/m^3 for 10 min 8.51 mg/m^3 for 30 min 6.39 mg/m^3 for 60 min 3.03 mg/m^3 for 4 h 2.63 mg/m^3 for 6 h
GB LC_{01}:	11.54 mg/m^3 for 10 min 5.84 mg/m^3 for 30 min 4.01 mg/m^3 for 60 min 2.09 mg/m^3 for 4 h 1.76 mg/m^3 for 6 h

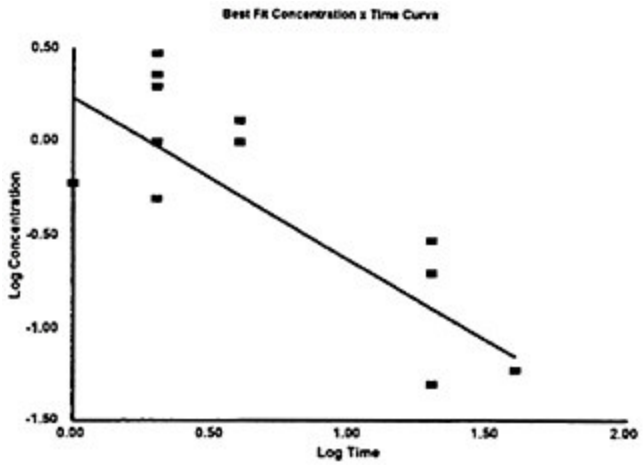
VX estimated LC_{01}:	2.89 mg/m^3 for 10 min 1.46 mg/m^3 for 30 min 1.00 mg/m^3 for 1 h 0.52 mg/m^3 for 4 h 0.44 mg/m^3 for 6 h (relative potency of 4 when compared with GB; see Section 4.3)
Scaling:	$C^n \times t = k$ (ten Berge et al. 1986). The n value of 2 used for agent GB is also used for agent VX (see text) to derive the 8-h AEGL-3 from the 6-h LC_{01}.
Uncertainty factors:	Interspecies: 3 (rat data) Intraspecies: 10 (adjustment for possible susceptible individuals)
Modifying factor (MF):	3 (for sparse VX data base)
Calculations:	$(C/[\text{uncertainty factors} \times \text{MF}])^2 \times t = k$
	$([0.44 \text{ mg/m}^3]/[10 \times 3 \times 3])^2 \times 6 \text{ h} = 1.16 \times 10^{-4} \text{ mg/m}^3 \times \text{h}$
10-min AEGL-3:	$(2.89 \text{ mg/m}^3)/100 = 0.029 \text{ mg/m}^3$
30-min AEGL-3:	$(1.46 \text{ mg/m}^3)/100 = 0.015 \text{ mg/m}^3$
1-h AEGL-3:	$(1.00 \text{ mg/m}^3)/100 = 0.010 \text{ mg/m}^3$
4-h AEGL-3:	$(0.52 \text{ mg/m}^3)/100 = 0.0052 \text{ mg/m}^3$
8-h AEGL-3:	$([1.16 \times 10^{-4} \text{ mg/m}^3]/8 \text{ h})^{1/2} = 0.0038 \text{ mg/m}^3$

APPENDIX B

Concentration-Time Curve for Sarin-Induced Miosis in Humans and Lethality in SD Rats

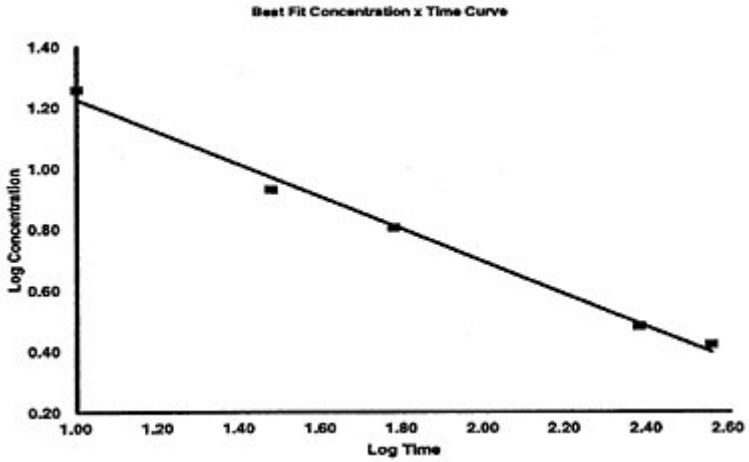
Data from Johns (1952), McKee and Woolcott (1949), and Baker and Sedgwick (1996) were used to assess the concentration-time relationship for agent GB-induced miosis

Time	Conc.	Log Time	Log Conc.	Regression Output:	
20	0.05	1.3010	−1.3010	Intercept	0.2371
2	0.5	0.3010	−0.3010	Slope	−0.8629
2	1	0.3010	0.0000	R Squared	0.6704
20	0.2	1.3010	−0.6990	Correlation	−0.8188
2	2	0.3010	0.3010	Observations	16
4	1	0.6021	0.0000		
2	2.3	0.3010	0.3617		
4	1.3	0.6021	0.1139		
20	0.3	1.3010	−0.5229		
2	3	0.3010	0.4771		
1	0.6	0.0000	−0.2218		
40	0.06	1.6021	−1.2218		
20	0.05	1.3010	−1.3010		
2	0.5	0.3010	−0.3010		
1	0.6	0.0000	−0.2218		
40	0.06	1.6021	−1.2218		
n=	1.16				
k=	1.88				



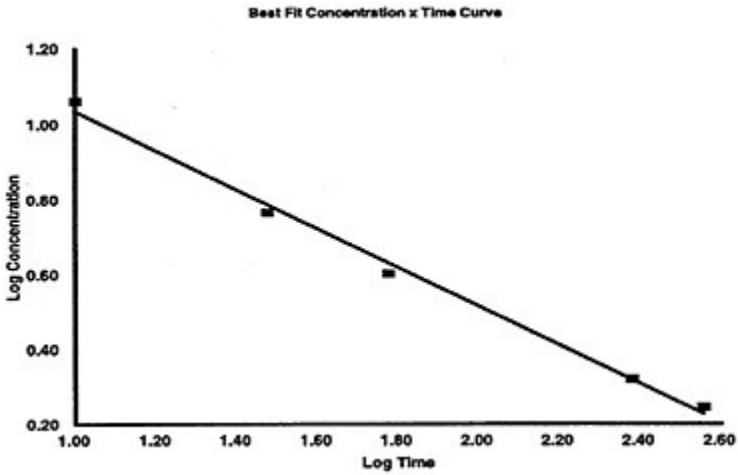
Data from Mioduszewski et al. (2000) were used to assess the concentration-time relationship for lethality (LC50) in female Sprague-Dawley rats exposed to GB vapor

Time	Conc.	Log Time	Log Conc.	Regression Output:	
10	18.1	1.0000	1.2577	Intercept	1.7577
30	8.51	1.4771	0.9299	Slope	−0.5325
60	6.39	1.7782	0.8055	R Squared	0.9927
240	3.03	2.3802	0.4814	Correlation	−0.9964
360	2.63	2.5563	0.4200	Observations	5
n=	1.88				
k=	2000.81				

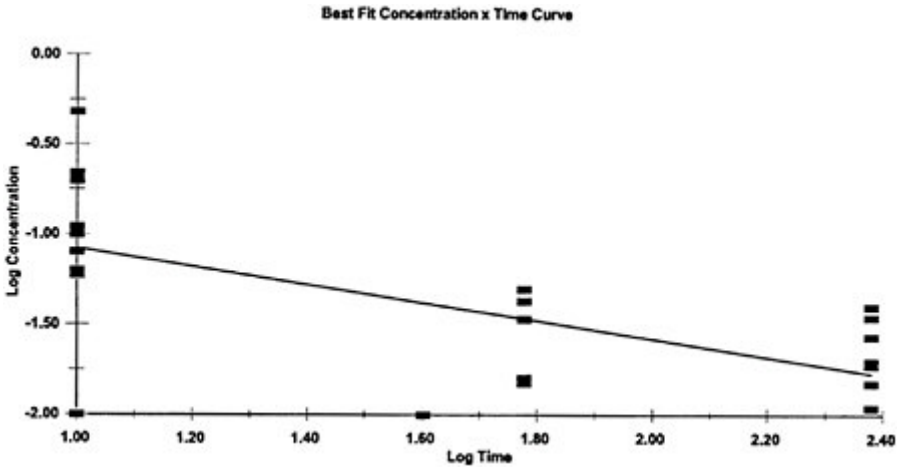


Data from Mioduszewski et al. (2000) were used to assess the concentration-time relationship for lethality (LC01) in female Sprague-Dawley rats exposed to GB vapor

Time	Conc.	Log Time	Log Conc.	Regression Output:	
10	11.54	1.0000	1.0622	Intercept	1.5532
30	5.84	1.4771	0.7664	Slope	−0.5188
60	4.01	1.7782	0.6031	R Squared	0.9948
240	2.09	2.3802	0.3201	Correlation	−0.9974
360	1.76	2.5563	0.2455	Observations	5
n=	1.93				
k=	986.04				



Chemical:		GB Vapor		
Study:		Mioduszewski et al., 2002b		
Species:		SD Rat		
Gender:		Female		
Time	Concentration	Log Time	Log Concentration	Regression Output
10	0.060	1.0000	−1.2218	Slope −0.5010
10	0.063	1.0000	−1.2007	R2 0.4335
10	0.080	1.0000	−1.0969	Correlation −0.6584
10	0.100	1.0000	−1.0000	Observations 24
10	0.110	1.0000	−0.9586	
10	0.200	1.0000	−0.6990	
10	0.220	1.0000	−0.6576	
10	0.480	1.0000	−0.3188	
40	0.010	1.6021	−2.0000	
60	0.015	1.7782	−1.8239	
60	0.016	1.7782	−1.7959	
60	0.034	1.7782	−1.4685	
60	0.043	1.7782	−1.3665	
60	0.050	1.7782	−1.3010	
240	0.011	2.3802	−1.9586	
240	0.011	2.3802	−1.9586	
240	0.015	2.3802	−1.8239	
240	0.015	2.3802	−1.8239	
240	0.019	2.3802	−1.7212	
240	0.020	2.3802	−1.6990	
240	0.027	2.3802	−1.5622	
240	0.035	2.3802	−1.4559	
240	0.040	2.3802	−1.3979	
n=	2.00			
k=	0.07			



APPENDIX C

Benchmark Exposure Analysis of GB Vapor Lethality Data for Female SD Rats

In August 2001, Mioduszewski et al. (2001) published their final report describing the experimental results of lethality studies in male and female adult SD rats exposed to varying Cts of GB vapor in a whole-body dynamic exposure chamber. Preliminary results of the experiments performed by Mioduszewski and his colleagues (documented in Mioduszewski et al. [2000]) were the basis for the AEGL-3 estimates submitted to the National Advisory Committee in 2000 that attained interim status.

A benchmark exposure calculation has been performed on the female rat 14-d vapor lethality data presented in the Mioduszewski et al. (2001) report, in accordance with guidance provided in the standing operating procedures (NRC 2001; Section 2.2.2.3.3). For comparison, a NumberCruncher Statistical System analysis has also been completed.

There appears to be some degree of controversy around using the benchmark dose approach for acute lethality data. The SOP workgroup will address this issue in the future.

There are eight models that accept dichotomous data in the benchmark dose (BMD) software package available on the EPA Web site(http://cfpub.epa.gov/ncea/cfm/bmds.cfm); gamma, logistic, log-log multistage, probit, log-probit, quantal-linear, quantal-quadratic, and Weibull. Evaluations were performed with all eight (multiplied by five time points, times the 5% response for the 95% lower confidence limit (LCL) and the 1% maximum likelihood estimate (MLE), as per the SOP). The Weibull and gamma programs would not run with the input data; contact with the EPA site Webmaster eventually revealed, through systems testing, that these two models require entry of a zero-concentration effect value in order to converge. The Mioduszewski et al. (2001) data set does not contain any zero-concentration effects data, and its addition would be an artificial alteration of the data set. It was concluded that the content of the Mioduszewski et al. (2001) data set is not compatible with requirements of the Weibull and gamma models; thus no analyses of the vapor lethality data were performed with these two models.

Tables C-1a,b and C-2 summarize the statistical results of this benchmark exposure analysis. Table C-1a,b is a summary of LC_{01} values obtained from all the BMD software routines; the ones on the low-er tier of the table (logistic, multistage, quantal-linear, quantal quadratic) are poor

TABLE C-1a Comparison of MINITAB Estimates of LC01 for Female Rat GB Vapor Inhalation Lethality with NumberCruncher Probit and BMD Analysis (14-d Lethality, Values in mg/m3)

Exposure Time	Basis of Interim AEGL (Mioduszewski et al. 2000)	MINITAB (Mioduszewski et al. 2001) (95% CI)	Number Cruncher Probit (SE)	BMD Probit (BMDL)	BMD Log Probit (no zero-concentration control) (BMDL)	BMD Log Probit (zero-concentration control) (BMDL)
10 min	11.54	11.56 (6.71–13.71	9.28 (all doses) (1.57) 10.48 (no low dose) (1.65)	10.77 (6.53)	11.56 (8.67)	11.56 (8.67)
30 min	5.84	5.62 (3.71–6.54)	5.27 (0.67)	4.74 (2.42)	5.62 (4.37)	5.62 (4.37)
60 min	4.01	4.23 (1.32–5.10)	4.05 (0.78)	3.76 (3.85; imprecise)	6.25 (5.51)	4.24 (2.24)
240 min (4 h)	2.09	1.77 (1.07–2.16)	1.59 (0.27)	1.33 (0.44)	1.77 (1.27)	1.77 (1.27)
360 min (6 h)	1.76	1.83 (1.04–2.12)	1.83 (0.21)	1.71 (1.54; imprecise)	2.41 (1.54)	1.83 (1.27)

TABLE C-1b Comparison of MINITAB Estimates of LC01 for Female Rat GB Vapor Inhalation Lethality with NumberCruncher Probit and BMD Analysis (14-d Lethality, Values in mg/m3)

Exposure Time	BMD Logistic (BMDL)	BMD Mulitstage (BMDL)	BMD Quantal Linear (BMDL)	BMD Quantal Quadratic (BMDL)
10 min	10.78 (7.53)	7.92 (1.51)	0.38 (0.26)	2.43 (1.99)
30 min	5.53 (4.07)	2.99 (0.43)	0.12 (0.09)	1.01 (0.86)
60 min	5.48 (3.54)	2.74 (0.12)	0.08 (0.06)	0.74 (0.63)
240 min (4 h)	1.61 (1.07)	1.07 (0.11)	0.03 (0.03)	0.33 (0.29)
360 min (6 h)	2.15 (1.30)	1.13 (0.07)	0.04 (0.03)	0.31 (0.27)

TABLE C-2 Comparison of AEGL-3 Values Calculated From Estimates of LC01 for Female Rat GB Vapor Inhalation Lethality (14-d Lethality, Values in mg/m3)a

Exposure Time	NAC Interim AEGL[b]	MINITAB Log Probit[c]	NumberCruncher Probit[b]	BMD Probit	BMD Log Probit[e]
10 min	0.38	0.39	0.31	0.36	0.39
30 min	0.19	0.19	0.18	0.16	0.19
60 min	0.13	0.14	0.13	0.13	0.21
240 min (4 h)	0.07	0.059	0.053	0.044	0.059
480 min (8 h)	0.051	0.053	0.053	0.049	0.070

[a]Interim values compared with those derived from Mioduszewski et al. (2001), MINITAB, NumberCruncher Probit, BMD Probit, and BMD Log Probit.

[b]Published in 66 Fed. Reg. 21947 (2001) and approved as interim values at NAC-21 on June 10–13, 2001. Derived from estimates of LC_{01} provided by Mioduszewski et al. from ongoing analysis of female rat lethality data documented in Mioduszewski et al. (2000).

[c]Derived from LC_{01} values provided in "Appendix A: Probit Analysis" of ECBC-TR-183, *ECBC Low-level Operational Toxicology Program; Phase 1—Inhalation Toxicity of Sarin Vapor in Rats as a Function of Exposure Concentration and Duration,* by Mioduszewski et al. (2001). These estimates of LC_{01} were generated by the probit analysis routine provided in Version 13 of MINITAB, a commercial statistical package offered by Minitab, Inc., of State College, Pennsylvania. The probit analysis in the reliability/survival section of the MINITAB package provides a log-probit analysis of the entered data. This log-probit analysis was duplicated during September 2001 in the Life Sciences Division of ORNL by entry of the female rat lethality data (14-d) from Table 3 (page 38) from ECBC-TR-183 into Release 13 (February 2000) of MINITAB. Performance of the BMD Log Probit analysis, with (forced) entry of a zero-concentration control, generated LC_{01} values equal to those generated by the log-probit MINITAB analysis (e.g., 10 min of 11.56 mg/m^3; 30 min of 5.62 mg/m^3 ; 60 min of 4.24 mg/m^3; 4 h of 1.77 mg/m^3; 6 h of 1.83 mg/m^3).

[d]NumberCruncher Statistical System Survival Analysis, Version 5.5 (copyright 1991, Dr. Jerry L Hintz, Kaysville, UT).

[e]No zero-concentration control.

Abbreviations: BMD, Benchmark Dose.

fits and are rejected from any further consideration. The first column following the exposure times is the set of MLE LC_{01} values used to develop the AEGL-3 estimates published in the *Federal Register* (66 Fed. Reg. 21940 [2001]) notice of May 2, 2001. The LC_{01} values in the second column following the exposure times are those published by Mioduszewski et al. (2001). All the remaining values presented in Table C-1a,b were based on the raw experimental data presented in Mioduszewski et al. (2001). The MINITAB log probit seems to be a reasonable fit with the lethality data. The experimental results on which this analysis is based are published in Mioduszewski et al. (2001, 2002a).

Because the statistical routines used to evaluate the data in Mioduszewski et al. (2000) differ slightly from those used in Mioduszewski et al. (2001), the LC_{01} values employed in developing interim AEGL-3 determinations also differ slightly. The resulting LC_{01} and AEGL-3 estimates developed with the same calculational approach, UFs, and *n* values as were applied in the AEGL-3 determinations presented earlier (see Appendix A), are summarized in tables C-1a,b and C-2. The *Federal Register* interim values for AEGL-3 (see Table C-2) are consistently lower or equal to the Mioduszewski et al. (2001) log-probit-derived estimates, with the single exception of the 4-h value. In the case of the 4-h value, the NAC interim AEGL-3 (0.070 mg/m^3) is somewhat greater than the 4-h AEGL-3 estimate derived from the Mioduszewski et al. (2001) log-probit derivation (0.059 mg/m^3; see Table C-2), a difference of 0.011 mg/m^3. The variation is slight.

The LC_{01} values presented in Mioduszewski et al. (2001), although slightly different from the preliminary results considered (Mioduszewski et al. 2000), represent a better documented and more widely accessible data set. These differences are acknowledged.

APPENDIX D

DERIVATION SUMMARY FOR ACUTE EXPOSURE GUIDELINE LEVELS FOR NERVE AGENTS

Derivation Summary For Agent GA (CAS No. 77–81–6) (Tabun; Dimethylamidocyanophosphate)

AEGL-1

10 min	30 min	1 h	4 h	8 h
0.0010 ppm (0.0069 mg/m^3)	0.00060 ppm (0.0040 mg/m^3)	0.00042 ppm (0.0028 mg/m^3)	0.00021 ppm (0.0014 mg/m^3)	0.00015 ppm (0.0010 mg/m^3)

Key reference: Mioduszewski, R.J., Manthei, J., Way, R., Burnett, D., Gaviola, B.Muse, W., Thomson, S., Sommerville, D., Crosier, R., Scotto, J., McCaskey, D., Crouse, C., and Matson, K. 2002b. Low-level sarin vapor exposure in rats: Effect of exposure concentration and duration on pupil size. ECBC-TR-235. Edgewood Chemical Biological Center, U.S. Army Soldier and Biological Chemical Command, Aberdeen Proving Ground, MD. (May 2002).

Secondary reference: (1) Van Helden, H.P.M.Trap, H.C, Kuijpers, W.C., Groen, B., Oostdijk, J.P., Vanwersch, R.A.P., Philippens, I.H.C., Langenberg, J.P. and Benschop, H.P. 2002. Low Level Exposure to GB Vapor in Air: Diagnosis/Dosimetry, Lowest Observable Effect Level, and Performance-Incapacitation. Research and Technology Organisation (RTO) Meeting Proceedings 75, Operational Medical Issues in Chemical and Biological Defense [RTO-MP-075, AC/323 (HFM-060) TP/37] held in Estoril, Portugal, 14–17 May 2001. North Atlantic Treaty Organisation, Research and Technology Organisation, BP 25, 7 Rue Ancelle, F-92201 Neuilly-sur-Seine CEDEX, France.
(2) Harvey, J.C. 1952. Clinical observations on volunteers exposed to concentrations of GB. Medical Laboratories Research Report No. 114, Publication Control No. 5030–114, MLCR 114 (CMLRE-ML-52). Army Chemical Center, MD.
(3) Johns, R.J. 1952. The effect of low concentrations of GB on the human eye. Chemical Corps Medical Laboratories Research Report No. 100, Publication Control

No. 5030–100 (CMLRE-ML-52), Army Chemical Center, MD.

Test species/strain/gender/number: Based on analysis from Section 4.3 of this document and Mioduszewski et al. (1998) that potency of GA is equal to that of agent GB for AEGL-1 effects (please see derivation for GB AEGL-1 derived from female Sprague-Dawley rat data).

Exposure route/concentrations/durations: Based on analysis from Section 4.3 and Mioduszewski et al. (1998) that potency of GA is equal to that of agent GB (please see derivation for GB AEGL-1) for AEGL-1 effects, and derived from GB vapor inhalation exposures for 10, 60, and 240 min in female SD rats.

Effects: Derivation of AEGL-1 values based on relative potency from Section 4.3 and Mioduszewski et al. (1998) study showingthat the relative potency of GA is equal to that of agent GB (please see derivation for GB AEGL-1) and from GB vapor exposure study of EC_{50} for miosis observed in adult female SD rats exposed to a range of GB vapor concentrations (0.01–0.48 mg/m^3) for 10 min (52 females), 60 min (35 females) and 240 min (55 females) (Mioduszewski et al. 2002b).

End point/concentration/rationale: Derivation of AEGL-1 values based on relative potency from Section 4.3 and Mioduszewski et al. (1998) study showing that the relative potency of GA is equal to that of agent GB (please see derivation for GB AEGL-1) for AEGL-1 effects and EC_{50} for miosis determination (a reversible, local, nondisabling, and transient effect) in the Mioduszewski et al. (2002b) study of young female SD rats exposed to agent GB. EC_{50} concentrations for miosis in female SD rats (the susceptible gender) are used as points of departure for AEGL-1 estimation. The miosis effects data of van Helden et al. (2001, 2002) (nonhuman primates), Harvey (1952) (human volunteers), and Johns (1952) (human volunteers) are supportive. The EC_{50} for miosis (postexposure pupil diameter 50% or less of the preexposure pupil diameter in 50% of exposed population) (Mioduszewski et al. 2002b) is not considered an adverse effect for humans.

Uncertainty factors/rationale: Based on relative potency from Section 4.3 and Mioduszewski et al. (1998) study showing that the relative potency GA is equal to that of agent GB (please see derivation for GB AEGL-1).
Total uncertainty factor: 10
Interspecies: 1—miosis response to GB vapor exposure is similar across multiple mammalian species.
Intraspecies: 10—for susceptible human subpopulations. Some individuals possess abnormally low levels of blood cholinesterase and carboxylesterase activity that may make them especially susceptible to the

effects of cholinesterase inhibitors such as nerve agents (see Section 4.5.3.). Therefore, a factor of 10 was retained.

Modifying factor: None (see derivation for agent GB).

Animal to human dosimetric adjustment: None applied.

Time scaling: Based on relative potency from Section 4.3 and Mioduszewski et al. (1998) study showing that potency of GA is equal to that of agent GB (please see derivation for GB AEGL-1) and $C^n \times t = k$ where n=2 and $k = 7.7 \times 10^{-6}$ mg/m^3×h for 10- to 30-min extrapolation, and $k = 5.8 \times 10^{-6}$ mg/m^3 ×h for 4-h to 8-h extrapolation for agent GB.

Data adequacy: Based on relative potency equal to that of agent GB (please see derivation for GB AEGL-1). The scarcity of dose-response data for agent GA forces the AEGL analysis to rely on assumptions of relative potency. Thus, AEGL values for agent GA are derivative. The relative potency assumptions for estimating AEGL-1 values for agent GA from the available database for agent GB need experimental confirmation.

AEGL-2

10 min	30 min	1 h	4 h	8 h
0.013 ppm (0.087 mg/m^3)	0.0075 ppm (0.050 mg/m^3)	0.0053 ppm (0.035 mg/m^3)	0.0026 ppm (0.017 mg/m^3)	0.0020 ppm (0.013 mg/m^3)

Key Reference: Baker, D.J., Sedgwick, E.M. 1996. Single fibre electromyographic changes in man after organophosphate exposure. Hum. Exp. Toxicol. 15:369–375.

Test species/strain/gender/number: Based on relative potency estimate from Section 4.3 of this document and Mioduszewski et al. (1998)—potency of GA is equal to that of agent GB for AEGL-2 effects (please see derivation for GB AEGL-2 derived from human volunteer data).

Exposure route/concentrations/durations: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998)—potency of GA is equal to that of agent GB for AEGL-2 effects (please see derivation for GB AEGL-2) and derived from GB vapor inhalation to humans in exposure chamber (GB at 0.5 mg/m^3 for 30 min while walking at a rate of 96 paces per min and breathing normally [Baker and Sedgwick 1996]).

Effects: Derivation of AEGL-2 values based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998) study showing that potency of GA is equal to that of agent GB for AEGL-2 effects (please see derivation for GB AEGL-2) and from GB vapor exposure study (Baker and Sedgwick 1996) in which observed effects included miosis in eight of e subjects, dyspnea and photophobia in some individuals, inhibition of RBC-ChE to approximately 60% (range of 54–66.1%) of individual baseline at 3 h and 3 d postexposure in eight of eight subjects, and small but measurable changes in single fibre electromyography (SFEMG) of the forearm that were detectable in the lab between 4 and 15 mo postexposure (but not detectable at 15–30 mo postexposure) in five of eight human volunteers exposed to GB at 0.5 mg/m^3 for 30 min. SFEMG changes considered subclinical. No permanent effects.

End point/concentration/rationale: Derivation of AEGL-2 values based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998) study showing that potency of GA is equal to that of agent GB for AEGL-2 effects (please see derivation for GB AEGL-2). Presence of miosis in all subjects exposed to GB vapor (Baker and Sedgwick 1996), in addition to the other observed signs in portions of the exposed population, indicates a greater level of effect than the EC_{50} considered for AEGL-1 determination; thus, there exists a heightened potential for reduced visual acuity following a 30-min exposure to GB at 0.5 mg/m^3. SFEMG effects are documented as long-lasting, but are subclinical and fully reversible. Such effects are not usually included as a basis for AEGL-2 estimation. However, due to the known steep

dose response for nerve agent vapor exposure, incorporation of the long-lasting SFEMG endpoint is here considered a protective interpretation of the AEGL-2 definition. The point of departure for AEGL-2 estimation is 0.5 mg GB/m^3 for 30 min.

Uncertainty factors/rationale: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998) study showing that potency of GA is equal to that of agent GB for AEGL-2 effects (please see derivation for GB AEGL-2).
Total uncertainty factor: 10
Interspecies: 1—human data
Intraspecies: 10—for possible susceptible human individuals. Some individuals possess abnormally low levels of blood cholinesterase and carboxylesterase activity that may make them especially susceptible to the effects of cholinesterase inhibitors such as nerve agents (see Section 4.5.3.). Therefore, a factor of 10 was retained.

Modifying factor: None (see derivation for agent GB).

Animal to human dosimetric adjustment: None applied (human data)

Time scaling: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998)—potency of GA is equal to that of agent GB for AEGL-2 effects (please see derivation for GB AEGL-2) and $C^n \times t = k$ where n=2 and k=0.0013 mg/m^3×h

Data adequacy: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998)—potency of GA is equal to that of agent GB for AEGL-2 effects (please see derivation for GB AEGL-2). The scarcity of dose-response data for agent GA forces the AEGL analysis to rely on assumptions of relative potency. Thus, AEGL values for agent GA are derivative. The relative potency assumptions for estimating AEGL-2 values for agent GA from the available database for agent GB need experimental confirmation.

AEGL-3

10 min	30 min	1 h	4 h	8 h
0.11 ppm (0.76 mg/m^3)	0.057 ppm (0.38 mg/m^3)	0.039 ppm (0.26 mg/m^3)	0.021 ppm (0.14 mg/m^3)	0.015 ppm (0.10 mg/m^3)

Key reference: (1) Mioduszewski, R.J., Manthei, J., Way, R., Burnett, D., Gaviola, B.Muse, W., Thomson, S., Sommerville, D. and Crosier, R. 2000. Estimating the probability of sarin vapor toxicity in rats as a function of exposure concentration and duration. Proceedings of the International Chemical Weapons Demilitarization Conference (CWD-2000), The Hague, NL. May 21–24, 2000.
(2) Mioduszewski, R.J., Manthei, J., Way, R., Burnett, D., Gaviola, B., Muse, W., Anthony, J., Durst, D., Sommerville, D., Crosier, R., Thomson, S., and Crouse, C. 2001. ECBC Low Level Operational Toxicology Program: Phase I—Inhalation toxicity of sarin vapor in rats as a function of exposure concentration and duration. ECBC-TR-183, Edgewood Research Development and Engineering Center, Aberdeen Proving Ground, MD. (August 2001).
(3) Mioduszewski, R.J., Manthei, J., Way, R., Burnett, D., Gaviola, B.Muse, W., Thomson, S., Sommerville, D., and Crosier, R. 2002a. Interaction of exposure concentration and duration in determining acute toxic effects of sarin vapor in rats. Toxicol. Sci. 66:176–184.

Test species/strain/gender/number: Based on relative potency estimate from Section 4.3 of this document and Mioduszewski et al. (1998)—potency of GA is approximately one-half that of agent GB for lethality (please see derivation for GB AEGL-3 derived from female Sprague-Dawley rat data).

Exposure route/concentrations/durations: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998)—potency of GA is approximately one-half that of agent GB for lethality (please see derivation for GB AEGL-3) and study of rat inhalation toxicity in dynamic mode exposure chamber (Mioduszewski et al. 2000, 2001, 2002a).

Effects: Derivation of AEGL-3 values based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998) study showing that potency of GA is approximately one-half that of agent GB for lethality (please see derivation for GB AEGL-3) and from GB vapor exposure study (Mioduszewski et al. 2000, 2001, 2002a) in which lethality and sublethal clinical signs monitored during and after exposure. Only lethality data reported at this time.

End point/concentration/rationale: Derivation of AEGL-3 values based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998) study showing that potency of GA is approximately one-half that of agent GB for lethality (please see derivation for GB AEGL-3) and from GB vapor exposure study (Mioduszewski et al. 2000, 2001, 2002a) which derived 14-d lethality estimates for female Sprague-Dawley rats (female rats were reported to be overall more sensitive to GB vapor toxicity than male rats over the range of exposure concentrations and durations studied). Gender differences in sensitivity are reported to be statistically significant at $p<0.01$ (Mioduszewski et al. 2000, 2001, 2002a). Probit analysis (MINITAB, version 13) presented in Mioduszewski et al. (2000) study provided 14-d LC_{50} and LC_{01} values for female rats. End point concentrations for LC_{01} reported for female SD rats (the susceptible gender) are used as points of departure from which to derive AEGL-3 estimates.

Uncertainty factors/rationale: Based on relative potency estimate from Mioduszewski et al. (1998) study showing that potency of GA is approximately one-half that of agent GB for lethality (please see derivation for GB AEGL-3)
Total uncertainty factor: 30
Interspecies: 3—The full default value of 10 was not considered necessary because the mechanism of toxicity in both rats and humans is the same, cholinesterase inhibition.
Intraspecies: 10—for possible susceptible human individuals. Some individuals possess abnormally low levels of blood cholinesterase and carboxlyesterase activity that may make them especially susceptible to the effects of cholinesterase inhibitors such as nerve agents (see Section 4.5.3). Therefore, a factor of 10 was retained.

Modifying factor: None (see derivation for agent GB).

Animal to Human Dosimetric Adjustment: None applied, insufficient data

Time scaling: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998)—potency of GA is approximately one-half that of agent GB for lethality (please see derivation for GB AEGL-3) and $C^n \times t = k$ where $n=2$ and $k=0.021$ mg/m^3×h to extrapolate from 6-h value to 8-h estimate of LC_{01}.

Data adequacy: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998) that potency of GA is approximately one-half that of agent GB for lethality (please see derivation for GB AEGL-3). The scarcity of dose-response data for agent GA forces the AEGL analysis to rely on assumptions of relative potency. Thus, AEGL values for agent GA are derivative. The relative potency assumptions for estimating AEGL-3 values

for agent GA from the available database for agent GB need experimental confirmation.

Derivation Summary for Agent GB (CAS No. 107–44–8) (Sarin; Isopropylmethylphosphonofluoridate)

AEGL-1

10 min	30 min	1 h	4 h	8 h
0.0012 ppm (0.0069 mg/m^3)	0.00068 ppm (0.0040 mg/m^3)	0.00048 ppm (0.0028 mg/m^3)	0.00024 ppm (0.0014 mg/m^3)	0.00017 ppm (0.0010 mg/m^3)

Key reference: Mioduszewski, R.J., Manthei, J., Way, R., Burnett, D., Gaviola, B.Muse, W., Thomson, S., Sommerville, D., Crosier, R., Scotto, J., McCaskey, D., Crouse, C., and Matson, K. 2002b. Low-level sarin vapor exposure in rats: Effect of exposure concentration and duration on pupil size. ECBC-TR-235. Edgewood Chemical Biological Center, U.S. Army Soldier and Biological Chemical Command, Aberdeen Proving Ground, MD. (May 2002).

Secondary reference: (1) van Helden, H.P.M.Trap, H.C, Kuijpers, W.C., Groen, B., Oostdijk, J.P., Vanwersch, R.A.P., Philippens, I.H.C., Langenberg, J.P. and Benschop, H.P. 2002. Low Level Exposure to GB Vapor in Air: Diagnosis/Dosimetry, Lowest Observable Effect Level, and Performance-Incapacitation. Research and Technology Organisation (RTO) Meeting Proceedings 75, Operational Medical Issues in Chemical and Biological Defense [RTO-MP-075, AC/323 (HFM-060) TP/37] held in Estoril, Portugal, 14–17 May 2001. North Atlantic Treaty Organisation, Research and Technology Organisation, BP 25, 7 Rue Ancelle, F-92201 Neuilly-sur-Seine CEDEX, France.
(2) Harvey, J.C. 1952. Clinical observations on volunteers exposed to concentrations of GB. Medical Laboratories Research Report No. 114, Publication Control No. 5030–114, MLCR 114 (CMLRE-ML-52). Army Chemical Center, MD.
(3) Johns, R.J. 1952. The effect of low concentrations of GB on the human eye. Chemical Corps Medical Laboratories Research Report No. 100, Publication Control No. 5030–100 (CMLRE-ML-52), Army Chemical Center, MD.

Test Species/Strain/Sex/Number: Rat, young adult (8–10 wk) female Sprague Dawley. Numbers of exposed individuals per time duration as follows: 10

min (52 females), 60 min (35 females) and 240 min (55 females) (Mioduszewski et al 2002b).

Exposure route/concentrations/durations: Whole body vapor exposure in 750-L dynamic airflow inhalation chamber to a range of GB vapor concentrations (0.01–0.48 mg/m^3) for 10 min, 60 min, and 240 min (Mioduszewski et al. 2002b).

Effects: Mioduszewski et al. (2002b) document EC_{50} for miosis observed in adult female SD rats exposed to a range of GB vapor concentrations (0.01– 0.48 mg/m^3) for durations of 10 min (52 females), 60 min (35 females), and 240 min (55 females). EC_{50} for miosis defined as a postexposure pupil diameter 50% or less of the preexposure pupil diameter in 50% of the exposed rat population (Mioduszewski et al. 2002b). EC_{50} for miosis is a reversible, local, and transient effect. No significant changes from baseline noted in blood RBC-ChE, BuChE, or carboxylesterase. No other clinical signs observed in the exposed rats.

End point/concentration/rationale: Mioduszewski et al. (2002b) document EC_{50} for miosis from their study of young female SD rats as a well-defined animal endpoint that is reversible, local, transient, and nondisabling. The EC_{50} concentrations for miosis in female SD rats (the susceptible gender) are used as points of departure for AEGL-1 estimation; the resulting AEGL-1 estimates are thus protective. The miosis effects data of van Helden et al. (2001, 2002) (nonhuman primates), Harvey (1952) (human volunteers), and Johns (1952) (human volunteers) are supportive. The EC_{50} for miosis effect (postexposure pupil diameter 50% or less of the preexposure pupil diameter in 50% of exposed population) (Mioduszewski et al. 2002b) is not considered an adverse effect for humans.

Uncertainty factors/rationale:
Total uncertainty factor: 10
Interspecies: 1—miosis response to GB vapor exposure is similar across multiple mammalian species.
Intraspecies: 10—for susceptible human subpopulations. Some individuals possess abnormally low levels of blood cholinesterase and carboxylesterase activity that may make them especially susceptible to the effects of cholinesterase inhibitors such as nerve agents (see Section 4.5.3). Therefore, a factor of 10 was retained.

Modifying factor: None. Strong arguments for not incorporating an additional modifying factor include the following:

1. Data are available for multiple species.
2. Data characterizing both lethal and nonlethal endpoints have been used in the analysis; these end points possess exposure-response data.
3. the mechanism of toxicity is known
4. the *n* value is derived from experimental data and is not the default
5. there are no uncertainties regarding reproductive and developmental effects or issues of carcinogenicity.

In consequence, no modifying factor was used in the estimation of AEGL-1 values.

Animal to human dosimetric adjustment: None applied.

Time scaling: $C^n \times t = k$ where n=2 and k=7.7×10^{-6} mg/m^3×h for 10- to 30-min extrapolation, and k=5.8×10^{-6} mg/m^3×h for 4-h to 8-h extrapolation. Current analyses based on a linear regression of the lethality and miosis data for female SD rats. The LC_{01} of GB to female Sprague-Dawley rats (Mioduszewski et al. 2000a,b, 2001, 2002a) yields an n value of 1.93 with an r^2 of 0.9948, while miosis (EC_{50} of GB to female Sprague-Dawley rats) (Mioduszewski et al 2002b) yields an n value of 2.00 with an r^2 of 0.4335 (see Appendix B). The anticholinesterase mechanism of mammalian toxicity for nerve agents is known, and all end points observed in human and animal studies represent a response continuum to anticholinesterase exposure. Accordingly, it is valid to apply an n value derived from compound-specific lethality data to time scaling for nonlethal effects, and an n value derived for miosis, to consideration of other toxic effects resulting from anticholinesterase exposure. This position is consistent with that of the recently published science policy of the EPA Office of Pesticide Programs (EPA 2000). Furthermore, this approach is preferable to use of default values. Therefore, n=2 is used as the scaling function for all AEGL time point derivations for agent GB.

Data adequacy: Confidence in the AEGL-1 values is high due to the robust data set. A total of 423 rats were used in this well-conducted study, of which 130 were controls. Three vapor exposure durations, each of which is an AEGL exposure interval (10, 60, and 240 min) were incorporated into the study design, and a sufficient number of individuals were exposed at each interval: 10 min (52 females), 60 min (35 females), and 240 min (55 females) (Mioduszewski et al. 2002b).

AEGL-2

10 min	30 min	1 h	4 h	8 h
0.015 ppm (0.087 mg/m^3)	0.0085 ppm (0.050 mg/m^3)	0.0060 ppm (0.035 mg/m^3)	0.0029 ppm (0.017 mg/m^3)	0.0022 ppm (0.013 mg/m^3)

Key reference: Baker, D.J., Sedgwick , E.M. 1996. Single fibre electromyographic changes in man after organophosphate exposure. Hum. Exp. Toxicol. 15:369–375.

Test species/strain/gender/number: human volunteers (healthy male servicemen), *N*=8 adults

Exposure route/concentrations/durations: Inhalation in exposure chamber; 0.5 mg/m^3 for 30 min while walking at a rate of 96 paces per minute and breathing normally.

Effects: Baker and Sedgwick (1996) document effects observed in human volunteers (0.5 mg/m^3 for 30 min while subjects in an exposure chamber walked at a rate of 96 paces per minute and breathed normally), including miosis in eight of eight subjects, dyspnea and photophobia in some individuals, inhibition of RBC-ChE to approximately 60% (range of 54– 66.1%) of individual baseline at 3 h and 3 d postexposure in eight of eight subjects, and small but measurable changes in single fibre electromyography (SFEMG) of the forearm that were detectable in the lab between 4 and 15 mo postexposure (in five of eight human volunteers), but not detectable at 15–30 mo postexposure. Respiratory effects resolved within minutes; ocular effects resolved within 48 h. SFEMG changes considered subclinical. No permanent effects.

End point/concentration/rationale: Presence of miosis in all subjects, in addition to other observed signs in portions of exposed population in the key study, indicates a greater level of effect than the EC_{50} considered for AEGL-1 determination; thus, there exists a heightened potential for reduced visual acuity following a 30 min exposure at 0.5 mg/m^3. SFEMG abnormalities detectable in the lab between 4 and 15 mo after a single experimental exposure documents these effects as long-lasting. Such subclinical and fully reversible effects are not usually included as a basis for AEGL-2 estimation. However, due to the known steep dose response for nerve agent vapor exposure, incorporation of the long-lasting SFEMG effect end point is here considered a protective interpretation of the AEGL-2 definition. The point of departure for AEGL-2 estimation is 0.5 mg/m^3 for 30 min.

Uncertainty factors/rationale:
Total uncertainty factor: 10
Interspecies: 1—human data

Intraspecies: 10—for possible susceptible human individuals. Some individuals possess abnormally low levels of blood cholinesterase and carboxylesterase activity that may make them especially susceptible to the effects of cholinesterase inhibitors such as nerve agents (see Section 4.5.3). Therefore, a factor of 10 was retained.

Modifying factor: None (see derivation for AEGL-1).

Animal to human dosimetric adjustment: None applied (human data)

Time scaling: $C^n \times t = k$ where n=2 and k=0.0013 mg/m^3×h. The recent data of Mioduszewki et al. (2000, 2001, 2002a,b) are determined to be the best source of an estimate for the n value for GB response (see also Appendix B and derivation for AEGL-1). The Mioduszewski et al. (2000, 2001, 2002a,b) data set is robust and compound-specific for the most completely characterized G-series nerve agent, agent GB. As outlined earlier, the mechanism of mammalian toxicity for nerve agents is known, and all end points observed in human and animal studies represent a response continuum to anticholinesterase exposure. Accordingly, it is valid to apply an n value derived from compound-specific miosis and lethality data to time scaling for all AEGL effects. This position is consistent with that of the recently published science policy of the EPA Office of Pesticide Programs (EPA 2000). Furthermore, this approach is preferable to use of default values. The current analysis is based on a linear regression of the lethality of GB to female Sprague-Dawley rats (Mioduszewski et al. 2000b), which yields an n value of 2.00 (see Appendix B). Therefore, n=2 is used as the scaling function for the AEGL-2 derivations.

Data adequacy: Confidence in the AEGL-2 values is limited by the lack of human experimental data for all AEGL-specific time frames and the lack of a clearly defined exposure-response relationship. The human data from which the AEGL-2 values were derived were limited by the short exposure time (30 min). Observed differences among human studies in identifying the toxicity threshold for low-dose exposures may be due to differences in individual sensitivities and breathing rates among the test populations, the steepness of the dose-response curve, and/or differences in experimental protocols or analytical methods.

AEGL-3

10 min	30 min	1 h	4 h	8 h
0.064 ppm (0.38 mg/m^3)	0.032 ppm (0.19 mg/m^3)	0.022 ppm (0.13 mg/m^3)	0.012 ppm (0.070 mg/m^3)	0.0087 ppm (0.051 mg/m^3)

Key reference: (1) Mioduszewski, R.J., Manthei, J., Way, R., Burnett, D., Gaviola, B.Muse, W., Thomson, S., Sommerville, D. and Crosier, R. 2000. Estimating the probability of sarin vapor toxicity in rats as a function of exposure concentration and duration. Proceedings of the International Chemical Weapons Demilitarization Conference (CWD-2000), The Hague, NL. May 21–24, 2000.
(2) Mioduszewski, R.J., Manthei, J., Way, R., Burnett, D., Gaviola, B., Muse, W., Anthony, J., Durst, D., Sommerville, D., Crosier, R., Thomson, S, and Crouse, C. 2001. ECBC Low Level Operational Toxicology Program: Phase I—Inhalation toxicity of sarin vapor in rats as a function of exposure concentration and duration. ECBC-TR-183, Edgewood Research Development and Engineering Center, Aberdeen Proving Ground, MD. (August 2001).
(3) Mioduszewski, R.J., Manthei, J., Way, R., Burnett, D., Gaviola, B.Muse, W., Thomson, S., Sommerville, D., and Crosier, R. 2002a. Interaction of exposure concentration and duration in determining acute toxic effects of sarin vapor in rats. Toxicol. Sci. 66:176–184.

Test species/strain/gender/number: Sprague-Dawley rats (8 to 10 wk old, from Charles River Laboratories), 10 females per concentration-time (Ct) combination, 50 females per time point,

Exposure route/concentrations/durations: Inhalation in dynamic mode exposure chamber; individuals exposed whole-body to one of five concentrations (2–56 mg/m^3) for one of seven exposure times (3, 10, 30, 60, 90, 240, or 360 min).

Effects: Mioduszewski et al. (2000, 2001, 2002a) document lethality and sublethal clinical signs monitored during and after experimental exposure. Only lethality data reported at this time.

End point/concentration/rationale: Mioduszewski et al. (2000, 2001, 2002a) document 14-d acute lethal toxicity of GB to female Sprague-Dawley rats. Female rats were reported to be more sensitive to GB vapor toxicity than males over the range of exposure concentrations and durations studied. Gender differences for lethality are reported by Mioduszewski et al. (2000, 2001, 2002a) to be statistically significant at $p<0.01$; thus, selection of

end point concentrations for LC_{01} reported for female SD rats (the susceptible gender) as points of departure from which to derive AEGL-3 estimates is protective. Probit analysis (MINITAB, version 13) presented in Mioduszewski et al. (2000) study gave the following 14-d LC_{50} and LC_{01} values for female rats:

LC_{50}
18.1 mg/m^3 for 10 min in female rats
8.51 mg/m^3 for 30 min in female rats.
6.39 mg/m^3 for 60 min in female rats
3.03 mg/m^3 for 4 h in female rats
2.63 mg/m^3 for 6 h in female rats

LC_{01}
11.54 mg/m^3 for 10 min in female rats
5.84 mg/m^3 for 30 min in female rats.
4.01 mg/m^3 for 60 min in female rats
2.09 mg/m^3 for 4 h in female rats
1.76 mg/m^3 for 6 h in female rats

Uncertainty factors/rationale:
Total uncertainty factor: 30
Interspecies: 3 (female rat data). The full default value of 10 was not considered necessary because the mechanism of toxicity in both rats and humans is the same, cholinesterase inhibition.
Intraspecies: 10—for possible susceptible human individuals. Some individuals possess abnormally low levels of blood cholinesterase and carboxylesterase activity that may make them especially susceptible to the effects of cholinesterase inhibitors such as nerve agents (see Section 4.5.3). Therefore, a factor of 10 was retained.

Modifying factor: None (see derivation for AEGL-1).

Animal to human dosimetric adjustment: None applied (insufficient data)

Time scaling: $C^n \times t = k$ where n=2 and k=0.021 mg/m^3×h. The recent data of Mioduszewski et al. (2000, 2001, 2002a,b) are determined to be the best source of an estimate for the n value for GB response (see also Appendix B and derivation for AEGL-1). The Mioduszewski et al. (2000, 2001, 2002a,b) data set is robust and compound-specific for the most completely characterized G-series nerve agent, agent GB. As outlined earlier, the mechanism of mammalian toxicity for nerve agents is known, and all endpoints observed in human and animal studies represent a response continuum to anticholinesterase exposure. Accordingly, it is valid to apply an

n value derived from compound-specific miosis and lethality data to time scaling for all AEGL effects. This position is consistent with that of the recently published science policy of the EPA Office of Pesticide Programs (EPA 2000). Furthermore, this approach is preferable to use of default values. The current analysis is based on a linear regression of the lethality of GB to female Sprague-Dawley rats (Mioduszewski et al. 2000), which yields an n value of 2.00 (see Appendix B). Therefore, n=2 is used as the scaling function for the AEGL-3 derivations. Time scaling was used to extrapolate from 6-h value provided by Mioduszewski et al. (2000) data to an 8-h estimate; scaling to derive 8-h LC_{01}. All other time-specific AEGL-3 values were derived directly from the LC_{01} values for female SD rats in the Mioduszewski et al. (2000) study.

Data adequacy: Further data analysis and experimentation is needed to more fully understand the degree of sensitivity to lethal exposure concentrations exhibited by female populations of test animals. Interspecies sensitivity could be more fully characterized by determining if similar results can be obtained under the same protocol with different test species (particularly nonhuman primates).

Derivation Summary for Agent GD (CAS No. 96–64–0) (Soman; Pinacolyl Methylphosphonofluoridate)

AEGL-1

10 min	30 min	1 h	4 h	8 h
0.00046 ppm (0.0035 mg/m^3)	0.00026 ppm (0.0020 mg/m^3)	0.00018 ppm (0.0014 mg/m^3)	0.000091 ppm (0.00070 mg/m^3)	0.000065 ppm (0.00050 mg/m^3)

Key reference: Mioduszewski, R.J., Manthei, J., Way, R., Burnett, D., Gaviola, B.Muse, W., Thomson, S., Sommerville, D., Crosier, R., Scotto, J., McCaskey, D., Crouse, C., and Matson, K. 2002b. Low-level sarin vapor exposure in rats: Effect of exposure concentration and duration on pupil size. ECBC-TR-235. Edgewood Chemical Biological Center, U.S. Army Soldier and Biological Chemical Command, Aberdeen Proving Ground, MD. (May 2002).

Secondary reference: (1) van Helden, H.P.M. Trap, H.C, Kuijpers, W.C., Groen, B., Oostdijk, J.P., Vanwersch, R.A.P., Philippens, I.H.C., Langenberg, J.P. and Benschop, H.P. 2002. Low Level Exposure to GB Vapor in Air: Diagnosis/Dosimetry, Lowest Observable Effect Level, and Performance-Incapacitation. Research and Technology Organisation (RTO) Meeting Proceedings 75, Operational Medical Issues in Chemical and Biological Defense [RTO-MP-075, AC/323 (HFM-060) TP/37] held in Estoril, Portugal, 14–17 May 2001. North Atlantic Treaty Organisation, Research and Technology Organisation, BP 25, 7 Rue Ancelle, F-92201 Neuilly-sur-Seine CEDEX, France.
(2) Harvey, J.C. 1952. Clinical observations on volunteers exposed to concentrations of GB. Medical Laboratories Research Report No. 114, Publication Control No. 5030– 114, MLCR 114 (CMLRE-ML-52). Army Chemical Center, MD.
(3) Johns, R.J. 1952. The effect of low concentrations of GB on the human eye. Chemical Corps Medical Laboratories Research Report No. 100, Publication Control No. 5030–100 (CMLRE-ML-52), Army Chemical Center, MD.

Test species/strain/gender/number: Based on relative potency from Section 4.3 of technical support document and Mioduszewski et al. (1998)—agent

GD is approximately twice as potent as agents GB and GA for AEGL-1 effects (please see derivation for GB AEGL-1 derived from rat data).

Exposure route/concentrations/durations: Based on relative potency from Section 4.3 and Mioduszewski et al. (1998)—agent GD is approximately twice as potent as agents GB and GA for AEGL-1 effects (please see derivation for GB AEGL-1) and derived from GB vapor inhalation exposures for 10, 60, and 240 min in female SD rats.

Effects: Derivation of AEGL-1 values based on relative potency from Section 4.3 and Mioduszewski et al. (1998) study showing that potency of GD is approximately twice as potent as agents GB and GA for AEGL-1 effects (please see derivation for GB AEGL-1) and from GB vapor exposure study of EC_{50} for miosis observed in adult female SD rats exposed to a range of GB vapor concentrations (0.01–0.48 mg/m^3) for 10 min (52 females), 60 min (35 females), and 240 min (55 females) (Mioduszewski et al. 2002b).

End point/concentration/rationale: Derivation of AEGL-1 values based on relative potency from Section 4.3 and Mioduszewski et al. (1998) study showing that potency of GD is approximately twice as potent as agents GB and GA for AEGL-1 effects (please see derivation for GB AEGL-1) and EC_{50} for miosis determination (a reversible, local, nondisabling and transient effect) in the Mioduszewski et al. (2002b) study of young female SD rats exposed to agent GB. The EC_{50} concentrations for miosis in female SD rats (the susceptible gender) are used as points of departure for AEGL-1 estimation. The miosis effects data of van Helden et al. (2001, 2002) (nonhuman primates), Harvey (1952) (human volunteers), and Johns (1952) (human volunteers) are supportive. The EC_{50} for miosis (postexposure pupil diameter 50% or less of the preexposure pupil diameter in 50% of exposed population) (Mioduszewski et al. 2002b) is not considered an adverse effect for humans.

Uncertainty factors/rationale: Based on relative potency from Section 4.3 and Mioduszewski et al. (1998) study showing that agent GD is approximately twice as potent as agents GB and GA for AEGL-1 effects (please see derivation for GB AEGL-1).
Total uncertainty factor: 10
Interspecies: 1—miosis response to GB vapor exposure is similar across multiple mammalian species.
Intraspecies: 10—for possible susceptible human individuals. Some individuals possess abnormally low levels of blood cholinesterase and carboxylesterase activity that may make them especially susceptible to the effects of cholinesterase inhibitors such as nerve agents (see Section 4.5.3). Therefore, a factor of 10 was retained.

Modifying factor: None (see derivation for agent GB).

Animal to human dosimetric adjustment: None applied

Time scaling: Based on relative potency from Section 4.3 and Mioduszewski et al. (1998)—agent GD is approximately twice as potent as agents GB and GA for AEGL-1 effects (please see derivation for GB AEGL-1) and $C^n \times t = k$ where n=2 and k=7.7×10^{-6} mg/m^3×h for 10-min to 30-min extrapolation and k=5.8×10^{-6} mg/m^3×h for 4-h to 8-h extrapolation for agent GB.

Data adequacy: Based on relative potency determination from Section 4.3 and Mioduszewski et al. (1998)—agent GD is approximately twice as potent as agents GB and GA for AEGL-1 effects (please see derivation for GB AEGL-1). The scarcity of dose-response data for agent GD forces the AEGL-1 analysis to rely on assumptions of relative potency. Thus, AEGL-1 values for agent GD are derivative. The relative potency assumptions for estimating AEGL-1 values for agent GD from the available database for agent GB need experimental confirmation.

AEGL-2

10 min	30 min	1 h	4 h	8 h
0.0057 ppm (0.044 mg/m^3)	0.0033 ppm (0.025 mg/m^3)	0.0022 ppm (0.018 mg/m^3)	0.0012 ppm (0.0085 mg/m^3)	0.00085 ppm (0.0065 mg/m^3)

Key reference: Baker, D.J., Sedgwick , E.M. 1996. Single fibre electromyographic changes in man after organophosphate exposure. Hum. Exp. Toxicol. 15:369–375.

Test species/strain/gender/number: Based on relative potency estimate from Section 4.3 of this document and Mioduszewski et al. (1998)—agent GD is approximately twice as potent as agents GB and GA for AEGL-2 effects (please see derivation for GB AEGL-2 derived from human volunteer data).

Exposure route/concentrations/durations: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998)—agent GD is approximately twice as potent as agents GB and GA for AEGL-2 effects (please see derivation for GB AEGL-2) and derived from GB vapor inhalation to humans in exposure chamber; 0.5 mg/m^3 for 30 min while walking at a rate of 96 paces per minute and breathing normally (Baker and Sedgwick 1996).

Effects: Derivation of AEGL-2 values based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998) study showing that agent GD is approximately twice as potent as agents GB and GA for AEGL-2 effects (please see derivation for GB AEGL-2) and from GB vapor exposure study (Baker and Sedgwick 1996) in which observed effects included miosis in eight of eight subjects, dyspnea and photophobia in some individuals, inhibition of RBC-ChE to approximately 60% (range of 54–66.1%) of individual baseline at 3 h and 3 d postexposure in eight of eight subjects, and small but measurable changes in single fibre electromyography (SFEMG) of the forearm that were detectable in the lab between 4 and 15 mo postexposure (but not detectable at 15–30 mo postexposure) in five of eight human volunteers exposed to GB at 0.5 mg/m^3 for 30 min. SFEMG changes considered subclinical. No permanent effects.

End point/concentration/rationale: Derivation of AEGL-2 values based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998) study showing that agent GD is approximately twice as potent as agents GB and GA for AEGL-2 effects (please see derivation for GB AEGL-2). Presence of miosis in all subjects exposed to GB vapor (Baker and Sedgwick 1996), in addition to other observed signs in portions of exposed population, indicates a greater level of effect than the EC_{50} considered for AEGL-1 determination; thus, there exists a heightened potential for reduced visual acuity following a 30-min exposure to 0.5 mg GB/m^3. SFEMG effects are documented as long-lasting, but are subclinical and fully reversible. Such

effects are not usually included as a basis for AEGL-2 estimation. However, due to the known steep dose response for nerve agent vapor exposure, incorporation of the long-lasting SFEMG end point is here considered a protective interpretation of the AEGL-2 definition. The point of departure for AEGL-2 estimation is 0.5 mg/m^3 for 30 min.

Uncertainty factors/rationale: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998) study showing that agent GD is approximately twice as potent as agents GB and GA for AEGL-2 effects (please see derivation for GB AEGL-2).
Total uncertainty factor: 10
Interspecies: 1 (human data)
Intraspecies: 10—for susceptible human subpopulations. Some individuals possess abnormally low levels of blood cholinesterase and carboxylesterase activity that may make them especially susceptible to the effects of cholinesterase inhibitors such as nerve agents (see Section 4.5.3). Therefore, a factor of 10 was retained.

Modifying factor: None (see derivation for agent GB).

Animal to human dosimetric adjustment: None applied (human data)

Time scaling: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998)—agent GD is approximately twice as potent as agents GB and GA for AEGL-2 effects (please see derivation for GB AEGL-2) and $C^n \times t = k$ where n=2 and k=0.0013 mg/m^3×h for agent GB.

Data adequacy: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998)—agent GD is approximately twice as potent as agents GB and GA for AEGL-2 effects (please see derivation for GB AEGL-2). The scarcity of dose-response data for agent GD forces the AEGL analysis to rely on assumptions of relative potency. Thus, AEGL values for agent GD are derivative. The relative potency assumptions for estimating AEGL-2 values for agent GD from the available database for agent GB need experimental confirmation.

AEGL-3

10 min	30 min	1 h	4 h	8 h
0.049 ppm (0.38 mg/m^3)	0.025 ppm (0.19 mg/m^3)	0.017 ppm (0.13 mg/m^3)	0.0091 ppm (0.070 mg/m^3)	0.0066 ppm (0.051 mg/m^3)

Key reference: (1) Mioduszewski, R.J., Manthei, J., Way, R., Burnett, D., Gaviola, B.Muse, W., Thomson, S., Sommerville, D. and Crosier, R. 2000. Estimating the probability of sarin vapor toxicity in rats as a function of exposure concentration and duration. Proceedings of the International Chemical Weapons Demilitarization Conference (CWD-2000), The Hague, NL. May 21–24, 2000.
(2) Mioduszewski, R.J., Manthei, J., Way R., et al. 2001. ECBC Low Level Operational Toxicology Program: Phase I—Inhalation toxicity of sarin vapor in rats as a function of exposure concentration and duration. ECBC-TR-183, Edgewood Research Development and Engineering Center, Aberdeen Proving Ground, MD. (August 2001).
(3) Mioduszewski, R.J., Manthei, J., Way, R., Burnett, D., Gaviola, B.Muse, W., Thomson, S., Sommerville, D., and Crosier, R. 2002a. Interaction of exposure concentration and duration in determining acute toxic effects of sarin vapor in rats. Toxicol. Sci. 66:176–184.
(4) Aas, P., Sterri, S.H., Hjermstad, H.P., Fonnum, F. 1985. A method for generating toxic vapors of soman: toxicity of soman by inhalation in rats. Toxicol. Appl. Pharmacol. 80:437–445. (Secondary study.)

Test species/strain/gender/number: Based on relative potency estimate from Section 4.3 of this document and Mioduszewski et al. (1998)—agent GD is equipotent to agent GB for lethality (please see derivation for GB AEGL-3 derived from female Sprague-Dawley rat data).

Exposure route/concentrations/durations: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998)—agent GD is equipotent to agent GB for lethality (please see derivation for GB AEGL-3) and study of rat inhalation toxicity in dynamic mode exposure chamber (Mioduszewski et al. 2000, 2001, 2002a).

Effects: Derivation of AEGL-3 values based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998) study showing that agent GD is equipotent to agent GB for lethality (please see derivation for GB AEGL-3) and from GB vapor exposure study (Mioduszewski et al. 2000, 2001, 2002a) in which lethality and sublethal clinical signs monitored during and after exposure. Only lethality data reported at this time.

End point/concentration/rationale: Derivation of AEGL-3 values based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998) study showing that agent GD is equipotent to agent GB for lethality (please see derivation for GB AEGL-3) and from GB vapor exposure study (Mioduszewski et al. 2000, 2001, 2002a) which derived 14-d lethality estimates for female Sprague-Dawley rats (female rats were reported to be overall more sensitive to GB vapor toxicity than male rats over the range of exposure concentrations and durations studied). Gender differences in sensitivity are reported to be statistically significant at $p<0.01$ (Mioduszewski et al. 2000, 2001, 2002a). Probit analysis (MINITAB, version 13) presented in Mioduszewski et al. (2000) study provided 14-d LC_{50} and LC_{01} values for female rats. End point concentrations for LC_{01} reported for female SD rats (the susceptible gender) are used as points of departure from which to derive AEGL-3 estimates.

Uncertainty factors/rationale: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998) study showing that agent GD is equipotent to agent GB for lethality (please see derivation for GB AEGL-3).
Total uncertainty factor: 30
Interspecies: 3 (female rat data). The full default value of 10 was not considered necessary because the mechanism of toxicity in both rats and humans is the same, cholinesterase inhibition. Intraspecies: 10—for possible susceptible human individuals. Some individuals possess abnormally low levels of blood cholinesterase and carboxylesterase activity that may make them especially suscep tible to the effects of cholinesterase inhibitors such as nerve agents (see Section 4.5.3). Therefore, a factor of 10 was retained.
From the secondary study of Aas et al. (1985)
Interspecies: 10 (rat data). Sparse data set for agent GD. Intraspecies: 10—for susceptible human subpopulations, and sparse data set for agent GD. Some individuals possess abnormally low levels of blood cholinesterase activity that may make them especially susceptible to the effects of cholinesterase inhibitors such as nerve agents (see Section 4.5.3. of technical support document). Therefore, a factor of 10 was retained.

Modifying factor: None (see derivation for agent GB).

Animal to human dosimetric adjustment: None applied (insufficient data).

Time scaling: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998) that agent GF is equipotent to agent GB for lethality (please see derivation for GB AEGL-3) and $C^n \times t = k$ where $n=2$ and $k=0.021$ mg/m^3×h to extrapolate from 6-h value to 8-h estimate of LC_{01}.

Data adequacy: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998)—agent GD is equipotent to agent GB for lethality (please see derivation for GB AEGL-3). The relative potency assumptions for estimating AEGL-3 values for agent GD from the available database for agent GB need experimental confirmation. The scarcity of dose-response data for agent GD forces the AEGL analysis to rely on assumptions of relative potency. Thus, AEGL-3 values for agent GD are derivative, with the exception of 10-min and 30-min values, which are confirmed by short-term experimental lethality data for Wistar rats as provided in a secondary study by Aas et al. (1985).

In the secondary study (Aas et al., 1985) of male Wistar rats (200–250 g), six animals tested at each of three exposure levels for periods of time <30 min by means of a dynamic inhalation chamber system; constant air concentration of GD at 21 mg/m^3 for undefined exposure periods (each less than 30 min). Aas et al. (1985) reported lethality and enzyme activity changes. Time scaling includes an n=2 and k=0.012 mg/m^3×h to derive 10-min and 30-min AEGL-3 estimates for agent GD. The Aas et al. (1985) data allow an estimate of the threshold for mortality in rats under this study protocol as equal to 335 mg·min/m^3 for an exposure period of 16 min. This lethal threshold is equivalent to an exposure to a GD concentration of 21 mg/m^3 for 16 min. Resulting AEGL-3 estimates (a 10-min AEGL-3 estimate of 0.27 mg/m^3 and a 30-min AEGL-3 estimate of 0.15 mg/m^3) are in good agreement with those derived by means of relative potency comparison with agent GB for the same time periods (see Appendix A for details of derivation).

Derivation Summary for Agent GF (CAS No. 329–99–7) (O-cyclohexyl-methylfluorophosphonate)

AEGL-1

10 min	30 min	1 h	4 h	8 h
0.00049 ppm (0.0035 mg/m^3)	0.00028 ppm (0.0020 mg/m^3)	0.00020 ppm (0.0014 mg/m^3)	0.00010 ppm (0.00070 mg/m^3)	0.000070 ppm (0.00050 mg/m^3)

Key reference: Mioduszewski, R.J., Manthei, J., Way, R., Burnett, D., Gaviola, B.Muse, W., Thomson, S., Sommerville, D., Crosier, R., Scotto, J., McCaskey, D., Crouse, C., and Matson, K. 2002b. Low-level sarin vapor exposure in rats: Effect of exposure concentration and duration on pupil size. ECBC-TR-235. Edgewood Chemical Biological Center, U.S. Army Soldier and Biological Chemical Command, Aberdeen Proving Ground, MD. (May 2002).

Secondary reference: (1) van Helden, H.P.M. Trap, H.C, Kuijpers, W.C., Groen, B., Oostdijk, J.P., Vanwersch, R.A.P., Philippens, I.H.C., Langenberg, J.P. and Benschop, H.P. 2002. Low Level Exposure to GB Vapor in Air: Diagnosis/Dosimetry, Lowest Observable Effect Level, and Performance-Incapacitation. Research and Technology Organisation (RTO) Meeting Proceedings 75, Operational Medical Issues in Chemical and Biological Defense [RTO-MP-075, AC/323 (HFM-060) TP/37] held in Estoril, Portugal, 14–17 May 2001. North Atlantic Treaty Organisation, Research and Technology Organisation, BP 25, 7 Rue Ancelle, F-92201 Neuilly-sur-Seine CEDEX, France.
(2) Harvey, J.C. 1952. Clinical observations on volunteers exposed to concentrations of GB. Medical Laboratories Research Report No. 114, Publication Control No. 5030–114, MLCR 114 (CMLRE-ML-52). Army Chemical Center, MD.
(3) Johns, R.J. 1952. The effect of low concentrations of GB on the human eye. Chemical Corps Medical Laboratories Research Report No. 100, Publication Control No. 5030–100 (CMLRE-ML-52), Army Chemical Center, MD.

Test species/strain/gender/number: Based on relative potency from Section 4.3 of this document and Mioduszewski et al. (1998)—agent GF is approxi

mately twice as potent as agents GB and GA for AEGL-1 effects (please see derivation for GB AEGL-1 derived from rat data).

Exposure route/concentrations/durations: Based on relative potency from Section 4.3 and Mioduszewski et al. (1998)—agent GF is approximately twice as potent as agents GB and GA for AEGL-1 effects (please see derivation for GB AEGL-1) and derived from GB vapor inhalation exposures for 10, 60, and 240 min in female SD rats.

Effects: Derivation of AEGL-1 values based on relative potency from Section 4.3 and Mioduszewski et al. (1998) study showing that agent GF is approximately twice as potent as agents GB and GA for AEGL-1 effects (please see derivation for GB AEGL-1) and from GB vapor exposure study of EC_{50} for miosis observed in adult female SD rats exposed to a range of GB vapor concentrations (0.01–0.48 mg/m^3) for 10 min (52 females), 60 min (35 females), and 240 min (55 females) (Mioduszewski et al. 2002b).

End point/concentration/rationale: Derivation of AEGL-1 values based on relative potency from Section 4.3 and Mioduszewski et al. (1998) study showing that agent GF is approximately twice as potent as agents GB and GA for AEGL-1 effects (please see derivation for GB AEGL-1) and EC_{50} for miosis determination (a reversible, local, nondisabling and transient effect) in the Mioduszewski et al. (2002b) study of young female SD rats exposed to agent GB. EC_{50} concentrations for miosis in female SD rats (the susceptible gender) are used as points of departure for AEGL-1 estimation. The miosis effects data of van Helden et al. (2001, 2002) (nonhuman primates), Harvey (1952) (human volunteers), and Johns (1952) (human volunteers) are supportive. The EC_{50} for miosis (postexposure pupil diameter 50% or less of the preexposure pupil diameter in 50% of exposed population) (Mioduszewski et al. 2002b) is not considered an adverse effect for humans.

Uncertainty factors/rationale: Based on relative potency from Section 4.3 and Mioduszewski et al. (1998) study showing that agent GF is approximately twice as potent as agents GB and GA for AEGL-1 effects (please see derivation for GB AEGL-1).
Total uncertainty factor: 10
Interspecies: 1—miosis response to GB vapor exposure is similar across multiple mammalian species.
Intraspecies: 10—for possible susceptible human individuals. Some individuals possess abnormally low levels of blood cholinesterase and carboxylesterase activity that may make them especially susceptible to the effects of cholinesterase inhibitors such as nerve agents (see Section 4.5.3). Therefore, a factor of 10 was retained.

Modifying factor: None (see derivation for agent GB).

Animal to human dosimetric adjustment: None applied (human data).

Time scaling: Based on relative potency from Section 4.3 and Mioduszewski et al. (1998)—agent GF is approximately twice as potent as agents GB and GA for AEGL-1 effects (please see derivation for GB AEGL-1) and $C^n \times t = k$ where $n=2$ and $k=7.7\times10^{-6}$ mg/m^3×h for 10- to 30-min extrapolation, and $k=5.8\times10^{-6}$ mg/m^3×h for 4-h to 8-h extrapolation for agent GB.

Data adequacy: Based on relative potency determination from Section 4.3 and Mioduszewski et al. (1998)—agent GF is approximately twice as potent as agents GB and GA for AEGL-1 effects (please see derivation for GB AEGL-1). The scarcity of dose-response data for agent GF forces the AEGL-1 analysis to rely on assumptions of relative potency. Thus, AEGL-1 values for agent GF are derivative. The relative potency assumptions for estimating AEGL-1 values for agent GF from the available database for agent GB need experimental confirmation.

AEGL-2

10 min	30 min	1 h	4 h	8 h
0.0062 ppm (0.044 mg/m^3)	0.0035 ppm (0.025 mg/m^3)	0.0024 ppm (0.018 mg/m^3)	0.0013 ppm (0.0085 mg/m^3)	0.00091 ppm (0.0065 mg/m^3)

Key reference: Baker, D.J., Sedgwick , E.M. 1996. Single fibre electromyographic changes in man after organophosphate exposure. Hum. Exp. Toxicol. 15:369–375.

Test species/strain/gender/number: Based on relative potency estimate from Section 4.3 of this document and Mioduszewski et al. (1998)—agent GF is approximately twice as potent as agents GB and GA for AEGL-2 effects (please see derivation for GB AEGL-2 derived from human volunteer data).

Exposure route/concentrations/durations: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998)—agent GF is approximately twice as potent as agents GB and GA for AEGL-2 effects (please see derivation for GB AEGL-2) and derived from GB vapor inhalation to humans in exposure chamber; GB at 0.5 mg/m^3 for 30 min while walking at a rate of 96 paces per minute and breathing normally (Baker and Sedgwick 1996).

Effects: Derivation of AEGL-2 values based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998) study showing that agent GF is approximately twice as potent as agents GB and GA for AEGL-2 effects (please see derivation for GB AEGL-2) and from GB vapor exposure study (Baker and Sedgwick 1996) in which observed effects included miosis in eight of eight subjects, dyspnea and photophobia in some individuals, inhibition of RBC-ChE to approximately 60% (range of 54–66.1%) of individual baseline at 3 h and 3 d postexposure in eight of eight subjects, and small but measurable changes in single fibre electromyography (SFEMG) of the forearm that were detectable in the lab between 4 and 15 mo postexposure (but not detectable at 15–30 mo postexposure) in five of eight human volunteers exposed to GB at 0.5 mg/m^3 for 30 min. SFEMG changes considered subclinical. No permanent effects.

End point/concentration/rationale: Derivation of AEGL-2 values based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998) study showing that agent GF is approximately twice as potent as agents GB and GA for AEGL-2 effects (please see derivation for GB AEGL-2). Presence of miosis in all subjects exposed to GB vapor (Baker and Sedgwick 1996), in addition to other observed signs in portions of exposed population, indicates a greater level of effect than the EC_{50} considered for AEGL-1 determination; thus there exists a heightened potential for reduced visual acuity following a 30-min exposure to GB at 0.5 mg/m^3. SFEMG effects are documented as long-lasting, but are subclinical and fully reversible. Such effects are not

usually included as a basis for AEGL-2 estimation. However, due to the known steep dose response for nerve agent vapor exposure, incorporation of the long-lasting SFEMG end point is here considered a protective interpretation of the AEGL-2 definition. The point of departure for AEGL- 2 estimation is 0.5 mg/m^3 for 30 min.

Uncertainty Factors/Rationale: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998) study showing that agent GF is approximately twice as potent as agents GB and GA for AEGL-2 effects (please see derivation for GB AEGL-2).
Total uncertainty factor: 10
Interspecies: 1 (human data).
Intraspecies: 10—for susceptible human subpopulations. Some individuals possess abnormally low levels of blood cholinesterase and carboxylesterase activity that may make them especially susceptible to the effects of cholinesterase inhibitors such as nerve agents (see Section 4.5.3). Therefore, a factor of 10 was retained.

Modifying factor: None (see derivation for agent GB).

Animal to human dosimetric adjustment: None applied (human data).

Time scaling: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998)—agent GF is approximately twice as potent as agents GB and GA for AEGL-2 effects (please see derivation for GB AEGL-2) and $C^n \times t = k$ where n=2 and k=0.0013 mg/m^3×h for agent GB.

Data adequacy: Based on relative potency estimate from Section 4.3 and Mioduszewski et al. (1998)—agent GF is approximately twice as potent as agents GB and GA for AEGL-2 effects (please see derivation for GB AEGL-2). The scarcity of dose-response data for agent GF forces the AEGL analysis to rely on assumptions of relative potency. Thus, AEGL values for agent GF are derivative. The relative potency assumptions for estimating AEGL-2 values for agent GF from the available database for agent GB need experimental confirmation.

AEGL-3

10 min	30 min	1 h	4 h	8 h
0.053 ppm (0.38 mg/m^3)	0.027 ppm (0.19 mg/m^3)	0.018 ppm (0.13 mg/m^3)	0.0098 ppm (0.070 mg/m^3)	0.0071 ppm (0.051 mg/m^3)

Key Reference: (1) Mioduszewski, R.J., Manthei, J., Way, R., Burnett, D., Gaviola, B.Muse, W., Thomson, S., Sommerville, D. and Crosier, R. 2000. Estimating the probability of sarin vapor toxicity in rats as a function of exposure concentration and duration. Proceedings of the International Chemical Weapons Demilitarization Conference (CWD-2000), The Hague, NL. May 21–24, 2000.
(2) Mioduszewski, R.J., Manthei, J., Way, R., Burnett, D., Gaviola, B., Muse, W., Anthony, J., Durst, D., Sommerville, D., Crosier, R., Thomson, S, and Crouse, C. 2001. ECBC Low Level Operational Toxicology Program: Phase I—Inhalation toxicity of sarin vapor in rats as a function of exposure concentration and duration. ECBC-TR-183, Edgewood Research Development and Engineering Center, Aberdeen Proving Ground, MD. (August 2001).
(3) Mioduszewski, R.J., Manthei, J., Way, R., Burnett, D., Gaviola, B.Muse, W., Thomson, S., Sommerville, D., and Crosier, R. 2002a. Interaction of exposure concentration and duration in determining acute toxic effects of sarin vapor in rats. Toxicol. Sci. 66:176–184.

Secondary reference: Anthony, J.S., Haley, M.V., Manthei, J.H., Way, R.A., Burnett, D.C., Gaviola, B.P., Sommerville, D.R., Crosier, R.B., Mioduszewski, R.J., Jakubouwski, E.M., Montgomery, J.L., Thomson, S.A. 2002. Inhalation toxicity of GF vapor in rats as a function of exposure concentration and duration and its potency comparison to GB. Late-breaking Poster presented at 41st Annual meeting of the Society of Toxicology, Nashville, TN (21 Mar 2002).

Test species/strain/gender/number: Based on relative potency estimate from Section 4.3 of this document and Mioduszewski et al. (1998)—agent GF is equipotent to agent GB for lethality (please see derivation for GB AEGL-3 derived from female Sprague-Dawley rat data). This assumption is supported by the recent study of Anthony et al. (2002).

Exposure route/concentrations/durations: Based on relative potency estimate from Section 4.3, Mioduszewski et al. (1998), and Anthony et al. (2002)—

agent GF is equipotent to agent GB for lethality (please see derivation for GB AEGL-3) and study of rat inhalation toxicity in dynamic mode exposure chamber (Mioduszewski et al. 2000, 2001, 2002a).

Effects: Derivation of AEGL-3 values based on relative potency estimate from Section 4.3 and the Mioduszewski et al. (1998) and Anthony et al (2002) studies showing that agent GF is equipotent to agent GB for lethality (please see derivation for GB AEGL-3) and from GB vapor exposure study (Mioduszewski, et al. 2000, 2001, 2002a) in which lethality and sublethal clinical signs monitored during and after exposure. Only lethality data reported at this time.

End point/concentration/rationale: Derivation of AEGL-3 values based on relative potency estimate from Section 4.3 and the Mioduszewski et al. (1998) and Anthony et al. (2002) studies showing that agent GF is equipotent to agent GB for lethality (please see derivation for GB AEGL-3) and from GB vapor exposure study (Mioduszewski et al. 2000, 2001, 2002a) which derived 14-d lethality estimates for female Sprague-Dawley rats (female rats were reported to be overall more sensitive to GB vapor toxicity than male rats over the range of exposure concentrations and durations studied). Gender differences in sensitivity are reported to be statistically significant at $p<0.01$ (Mioduszewski et al. 2000, 2001, 2002a). End point concentrations for LC_{01} reported for female SD rats (the susceptible gender) are used as points of departure from which to derive AEGL-3 estimates. Probit analysis (MINITAB, version 13) presented in the Mioduszewski et al. (2000) study provided 14-d LC_{50} and LC_{01} values for female rats.

Uncertainty factors/rationale: Based on relative potency estimate from Section 4.3 and the Mioduszewski et al. (1998), and Anthony et al. (2002) studies showing that agent GF is equipotent to agent GB for lethality (please see derivation for GB AEGL-3).
Total uncertainty factor: 30
Interspecies: 3 (female rat data). The full default value of 10 was not considered necessary because the mechanism of toxicity in both rats and humans is the same, cholinesterase inhibition.
Intraspecies: 10—for possible susceptible human individuals. Some individuals possess abnormally low levels of blood cholinesterase and carboxylesterase activity that may make them especially susceptible to the effects of cholinesterase inhibitors such as nerve agents (see Section 4.5.3.). Therefore, a factor of 10 was retained.

Modifying factor: None (see derivation for agent GB).

Time scaling: Based on relative potency estimate from Section 4.3 of this document, Mioduszewski et al. (1998), and Anthony et al. (2002)—agent GF

is equipotent to agent GB for lethality (please see derivation for GB AEGL-3) and $C^n \times t = k$ where n=2 and k=0.021 mg/m^3×h to extrapolate from 6-h value to 8-h estimate of LC_{01}.

Data adequacy: Based on relative potency estimate from Section 4.3, Mioduszewski et al. (1998), and Anthony et al. (2002)—agent GF is equipotent to agent GB for lethality (please see derivation for GB AEGL-3). The scarcity of dose-response data for agent GF forces the AEGL analysis to rely on assumptions of relative potency. Thus, AEGL-3 values for agent GF are derivative. The relative potency assumptions for estimating AEGL-3 values for agent GF from the available database for agent GB need experimental confirmation.

Derivation Summary for Agent VX Vapor (CAS No. 50782–69–9) (O-Ethyl-S-(Isopropyl-Aminoethyl)Methyl Phosphonothiolate)

AEGL-1

10 min	30 min	1 h	4 h	8 h
0.000052 ppm (0.00057 mg/m^3)	0.000030 ppm (0.00033 mg/m^3)	0.000016 ppm (0.00017 mg/m^3)	0.0000091 ppm (0.00010 mg/m^3)	0.0000065 ppm (0.000071 mg/m^3)

Key reference: Mioduszewski et al. (2002b). Low-level sarin vapor exposure in rats: Effect of exposure concentration and duration on pupil size (ECBC-TR-235). Edgewood Chemical Biological Center, U.S. Army Soldier and Biological Chemical Command, Aberdeen Proving Ground, MD.

Secondary references: (1) Grob, D., and Harvey, J.C. 1958. Effects in man of the anticholinesterase compound Sarin (isopropyl methyl phosphonofluoridate). J. Clin. Invest. 37:350–368
(2) Sidell, F.R., and Groff, W.A. 1974. The reactivatibility of cholinesterase inhibited by VX and sarin in man. Toxicol. Appl. Pharmacol. 27:241–252.

Test species/strain/gender/number: Based on relative potency from Grob and Harvey (1958) and Sidell and Groff (1974)—potency of agent VX is approximately 4 times that of agent GB for AEGL-1 effects (please see derivation for GB AEGL-1, derived from female Sprague-Dawley rat data for EC_{50} miosis).

Exposure route/concentrations/durations: Based on relative potency from Grob and Harvey (1958) and Sidell and Groff (1974)—potency of agent VX is approximately 4 times that of agent GB for AEGL-1 effects (please see derivation for GB AEGL-1) and derived from GB vapor exposures to female Sprague-Dawley rats for EC_{50} miosis at 10, 60, and 240 min

Effects: Derivation of AEGL-1 values based on relative potency from Grob and Harvey (1958) and Sidell and Groff (1974) studies showing that potency of agent VX is approximately 4 times that of agent GB for AEGL-1 effects (please see derivation for GB AEGL-1) and VX estimates derived from data in GB vapor exposure study (Miodiszewski et al. 2002b) in which EC_{50} for miosis (a postexposure pupil diameter 50% or less of the preexposure pupil diameter in 50% of the exposed rat population) in female SD rats was determined for 10, 60, and 240 min. Estimated concentrations of VX

expected to produce similar effects are 0.017 mg/m^3 for 10 min, 0.005 mg/m^3 for 60 min, and 0.003 mg/m^3 for 240 min.

End point/concentration/rationale: Derivation of AEGL-1 values based on relative potency from Grob and Harvey (1958) and Sidell and Groff (1974) studies showing that potency of agent VX is approximately 4 times that of agent GB (please see derivation for GB AEGL-1) and determination that the EC_{50} for miosis is based on a well-defined animal end point in a susceptible gender and is reversible, local, nondisabling, and transient (Mioduszewski et al. [2002b] study of young female SD rats exposed to GB). Estimated concentrations of VX expected to produce similar effects are 0.017 mg/m^3 for 10 min, 0.005 mg/m^3 for 60 min, and 0.003 mg/m^3 for 240 min; selection of these estimated EC_{50} concentrations for female SD rats (the susceptible gender) as the points of departure for agent VX AEGL-1 estimation is thus protective. The miosis effects data of van Helden et al. (2001, 2002) (nonhuman primates), Harvey (1952) (human volunteers), and Johns (1952) (human volunteers) are supportive. The EC_{50} for miosis (a postexposure pupil diameter 50% or less of the preexposure pupil diameter in 50% of the exposed rat population) (Mioduszewski et al. 2002b) is not considered an adverse effect for humans. The AEGL values are estimates for VX vapor exposures only.

Uncertainty factors/rationale: Based on relative potency from Grob and Harvey (1958) and Sidell and Groff (1974) studies showing that potency of agent VX is approximately 4 times that of agent GB (please see derivation for GB AEGL-1).
Total uncertainty factor: 30
Interspecies: 1—miosis response to nerve agent vapor exposure is similar across mammalian species (as for agent GB AEGL-1 estima tion).
Intraspecies: 10—for possible susceptible human individuals. Some individuals possess abnormally low levels of blood cholinesterase activity that may make them especially susceptible to the effects of cholinesterase inhibitors such as nerve agents (Morgan 1989; Wills 1972; Opresko et al. 1998). Therefore, a factor of 10 was retained.

Modifying factor: 3 (for sparse VX database).

Animal to human dosimetric adjustment: None applied (human data).

Time scaling: Based on relative potency from Grob and Harvey (1958) and Sidell and Groff (1974)—potency of agent VX is approximately 4 times that of agent GB (please see derivation for GB AEGL-1) and $C^n \times t = k$ where $n = 2$, and $k = 5.0 \times 10^{-8}$ mg/m^3×h for 10 to 30 min extrapolation, and $k = 4.0 \times 10^{-8}$ mg/m^3×h for 4-h to 8-h extrapolation.

Data adequacy: Based on relative potency—potency of agent VX is approximately 4 times that of agent GB (please see derivation for GB AEGL-1). The scarcity of dose-response data for agent VX forces the AEGL analysis to rely on assumptions of relative potency. Thus, AEGL values for agent VX are derivative. The relative potency assumptions for estimating AEGL-1 values for agent VX from the available database for agent GB need experimental confirmation.

AEGL-2

10 min	30 min	1 h	4 h	8 h
0.00065 ppm (0.0072 mg/m^3)	0.00038 ppm (0.0042 mg/m^3)	0.00027 ppm (0.0029 mg/m^3)	0.00014 ppm (0.0015 mg/m^3)	0.000095 ppm (0.0010 mg/m^3)

Key reference: Baker, D.J. and Sedgwick, E.M., 1996. Single-fibre electromyographic changes in man after organophosphate exposure. Hum. Exp. Toxicol. 15:369–375.

Secondary references: Grob, D., and Harvey, J.C. 1958. Effects in man of the anticholinesterase compound Sarin (isopropyl methyl phosphonofluoridate). J. Clin. Invest. 37:350–368; Sidell, F.R., and Groff, W.A. 1974. The reactivatibility of cholinesterase inhibited by VX and sarin in man. Toxicol. Appl. Pharmacol. 27:241–252.

Test species/strain/gender/number: Based on relative potency from Grob and Harvey (1958) and Sidell and Groff (1974)—potency of agent VX is approximately 4 times that of agent GB for AEGL-2 effects (please see derivation for GB AEGL-2 derived from human volunteer data).

Exposure route/concentrations/durations: Based on relative potency from Grob and Harvey (1958) and Sidell and Groff (1974)—potency of agent VX is approximately 4 times that of agent GB (please see derivation for GB AEGL-2) and derived from GB vapor inhalation to humans in exposure chamber; 0.5 mg/m^3 for 30 min while walking at a rate of 96 paces per minute and breathing normally (Baker and Sedgwick 1996).

Effects: Derivation of AEGL-2 values based on relative potency from Grob and Harvey (1958) and Sidell and Groff (1974) studies showing that potency of agent VX is approximately 4 times that of agent GB for AEGL-2 effects (please see derivation for GB AEGL-2). VX estimates derived from GB vapor exposure study (Baker and Sedgwick 1996) in which observed effects included miosis in eight of eight subjects, dyspnea and photophobia in some individuals, inhibition of RBC-ChE to approximately 60% of individual baseline at 3 h and 3 d postexposure in eight of eight subjects, and small but measurable changes in single fibre electromyography (SFEMG) of the forearm that were detectable in the lab between 4 and 15 mo postexposure (but not detectable at 15–30 mo postexposure) in five of eight human volunteers exposed to GB at 0.5 mg/m^3 for 30 min. SFEMG changes considered subclinical. No permanent effects.

End point/concentration/rationale: Derivation of AEGL-2 values based on relative potency from Grob and Harvey (1958) and Sidell and Groff (1974)

studies showing that potency of agent VX is approximately 4 times that of agent GB for AEGL-2 effects (please see derivation for GB AEGL-2) and determination that a 30-min exposure to GB at 0.5 mg/m^3 results in a greater level of effect than the EC_{50} considered for AEGL-1 determination (see Baker and Sedgwick [1996]); thus, there exists a heightened potential for reduced visual acuity following experimental exposures. Estimated concentration of VX expected to produce similar effects is 0.125 mg/m^3 for 30 min; this concentration is the point of departure for agent VX AEGL- 2 estimations. Inclusion of the long-lasting but subclinical and fully reversible SFEMG effect is here considered a protective interpretation of the AEGL-2 definition, given the known steep dose response for nerve agent vapor exposure. The AEGL values are estimates for VX vapor exposures only.

Uncertainty Factors/Rationale: Based on relative potency from Grob and Harvey (1958) and Sidell and Groff (1974) studies showing that potency of agent VX is approximately 4 times that of agent GB for AEGL-2 effects (please see derivation for GB AEGL-2).
Total uncertainty factor: 30
Interspecies: 1 (human data).
Intraspecies: 10—for possible susceptible human individuals. Some individuals possess abnormally low levels of blood cholinesterase activity that may make them especially susceptible to the effects of cholinesterase inhibitors such as nerve agents (Morgan 1989; Wills 1972; Opresko et al., 1998). Therefore, a factor of 10 was retained.

Modifying factor: 3 (for sparse VX database).

Animal to human dosimetric adjustment: None applied (human data)

Time scaling: Based on relative potency from Grob and Harvey (1958) and Sidell and Groff (1974)—potency of agent VX is approximately 4 times that of agent GB for AEGL-2 effects (please see derivation for GB AEGL-2) and $C^n \times t = k$ where $n=2$ and $k=8.6\times10^{-6}$ mg/m$^3\times$h.

Data adequacy: Based on relative potency estimate from Grob and Harvey (1958) and Sidell and Groff (1974)—potency of agent VX is approximately 4 times that of agent GB for AEGL-2 effects (please see derivation for GB AEGL-2). The scarcity of dose-response data for agent VX forces the AEGL analysis to rely on assumptions of relative potency. Thus, AEGL values for agent VX are derivative. The relative potency assumptions for estimating AEGL-2 values for agent VX from the available database for agent GB need experimental confirmation.

AEGL-3

10 min	30 min	1 h	4 h	8 h
0.0027 ppm (0.029 mg/m^3)	0.0014 ppm (0.015 mg/m^3)	0.00091 ppm (0.010 mg/m^3)	0.00048 ppm (0.0052 mg/m^3)	0.00035 ppm (0.0038 mg/m^3)

Key reference: Mioduszewski et al. 2002a. Interaction of exposure concentration and duration in determining acute toxic effects of sarin vapor in rats. Toxicol. Sci. 66:176–184.

Secondary references: (1) Grob, D., and Harvey, J.C. 1958. Effects in man of the anticholinesterase compound Sarin (isopropyl methyl phosphonofluoridate). J. Clin. Invest. 37:350–368.
(2) Sidell, F.R., and Groff, W.A. 1974. The reactivatibility of cholinesterase inhibited by VX and sarin in man. Toxicol. Appl. Pharmacol. 27:241–252.

Test species/strain/gender/number: Based on assumptions previously stated from Grob and Harvey (1958) and Sidell and Groff (1974)—potency of agent VX is approximately 4 times that of agent GB (please see derivation for GB AEGL-3 derived from female Sprague-Dawley rat data) and RBC-ChE$_{50}$ is part of an anticholinesterase response continuum.

Exposure route/concentrations/durations: Based on assumption previously stated from Grob and Harvey (1958) and Sidell and Groff (1974)—that the potency of agent VX is approximately 4 times that of agent GB (please see derivation for GB AEGL-3) and the study of rat inhalation toxicity in dynamic mode exposure chamber (Mioduszewski et al. 2000, 2001, 2002a).

Effects: Derivation of AEGL-3 values based on assumption previously stated from Grob and Harvey (1958) and Sidell and Groff (1974) studies showing that potency of agent VX is approximately 4 times that of agent GB (please see derivation for GB AEGL-3) and from GB vapor exposure study (Mioduszewski et al. 2000, 2001, 2002a) in which lethality and sublethal clinical signs were monitored during and after exposure. Only lethality data reported at this time.

End point/concentration/rationale: Derivation of AEGL-3 values based on assumption previously stated from Grob and Harvey (1958) and Sidell and Groff (1974) studies showing that potency of agent VX is approximately 4 times that of agent GB (please see derivation for GB AEGL-3) and from GB vapor exposure study (Mioduszewski et al. 2000, 2001, 2002a) of female SD rats exposed to GB vapor. Gender differences for lethality are significant. The LC_{01} values for GB exposure were multiplied by a factor of 0.25 to estimate the LC_{01} value for agent VX. These estimated concentrations for VX

LC_{01} in female SD rats (the susceptible gender) are the points of departure for agent VX AEGL-3 estimations, which are thus protective. The AEGL values are estimates for VX vapor exposures only.

Uncertainty factors/rationale: Based on assumption previously stated from Grob and Harvey (1958) and Sidell and Groff (1974) studies showing that potency of agent VX is approximately 4 times that of agent GB (please see derivation for GB AEGL-3).
Total uncertainty factor: 100
Interspecies: 3—female rat data. The full default value of 10 is not considered appropriate because the mechanism of toxicity in lab rodents and humans is ChE inhibition.
Intraspecies: 10—for possible susceptible human individuals. Some individuals possess abnormally low levels of blood cholinesterase and carboxylesterase activity that may make them especially susceptible to the effects of cholinesterase inhibitors such as nerve agents (Morgan 1989; Wills 1972; Opresko et al. 1998). Therefore, a factor of 10 was retained.

Modifying factor: 3 (for sparse VX database).

Animal to human dosimetric adjustment: None applied (insufficient data)

Time scaling: $C^n \times t = k$ where n=2 and k=1.^{1}6×10^{-4} mg/m3×h, based on the assumption that the scaling function for agent VX is similar to that derived for agent GB from the experimental rat data of Mioduszewski et al. (2000, 2001, 2002a,b). Extrapolation from 6-h experimental value to 8-h AEGL-3.

Data adequacy: The scarcity of dose-response data for agent VX forces the AEGL analysis to rely on assumptions of relative potency. Thus, AEGL values for agent VX are derivative. The relative potency assumptions for estimating AEGL-3 values for agent VX from the available database for agent GB need experimental confirmation.

2

Sulfur Mustard (Agent HD)[1]

SUMMARY

Sulfur mustard (agent HD) is an alkylating chemical vesicant that affects any epithelial surface it comes in contact with; it has been developed and used as a warfare agent. The active component is bis(2-chloroethyl)sulfide (CAS Registry No. 505–60–2). Although the chemical is a liquid at ordinary ambient temperatures, its volatility results in rapid generation of vapors that have a garlic-like odor. Due to its low aqueous solubility, it is

[1]This document was prepared by the AEGL Development Team comprising Robert Young (Oak Ridge National Laboratory) and Kenneth Still (Chemical Manager) of the National Advisory Committee (NAC) on Acute Exposure Guideline Levels for Hazardous Substances. The NAC reviewed and revised the document and the AEGL values as deemed necessary. Both the document and the AEGL values were then reviewed by the National Research Council (NRC) Subcommittee on Acute Exposure Guideline Levels. The NRC subcommittee concludes that the AEGLs developed in this document are scientifically valid conclusions based on the data reviewed by the NRC and are consistent with the NRC guidelines reports (NRC 1993, 2001).

persistent in the environment. Odor thresholds of 1 mg·min/m^3, 0.15 mg/m^3, and 0.6 mg/m^3 have been reported. Among various U.S. Army facilities, there are currently approximately 17,018.1 tons of sulfur mustard (agent HD) awaiting disposal.

Exposure to sulfur mustard vapor may result in irritation and damage to the eyes, respiratory tract, and skin. The toxic effects of sulfur mustard are temperature- and humidity-dependent; for a given exposure, the effects could be greater with increasing temperature and humidity. An exposure-dependent latency period of hours to days is documented and is relevant for all routes of exposure but may be shorter for ocular and upper respiratory tract effects than for dermal and systemic responses. Both human and animal data indicate that the eyes are the most sensitive organ/tissue; deaths resulting from sulfur mustard exposure are more often the result of respiratory tract involvement. Because the toxic effects of sulfur mustard (at least for short time periods) appear to be a linear function of exposure duration and exposure concentration, most of the available exposure-response data are expressed as cumulative exposures (Ct).

Minor ocular irritation (conjunctival injection in the absence of irritation) occurs in humans following exposure at 12–30 mg·min/m^3. More severe effects develop at 60–75 mg·min/m^3 (conjunctivitis, irritation, photophobia) and at 100 mg·min/m^3 (severe ocular irritation). Vapor inhalation LCt_{50} estimates for humans range from 900 mg·min/m^3 to 1,500 mg·min/m^3.

Animal lethality following acute exposure to sulfur mustard occurs at cumulative exposures ranging from approximately 600 mg·min/m^3 to 1,500 mg·min/m^3. Nonlethal effects were similar to those observed in humans and included effects on the eyes, respiratory tract, and skin. Long-term exposure of dogs, rats, and guinea pigs to concentrations at 0.03 mg/m^3 produced only minor signs of ocular and respiratory tract irritation. One-hour (h) exposure of mice to concentrations up to 16.9 mg/m^3 resulted in notable effects on respiratory parameters, and acute exposures of rabbits (20 minutes [min]) to 12 h) to concentrations ranging from 58 mg/m^3 to 389 mg/m^3 (Ct ≥2,300 mg·min/m^3) resulted in severe respiratory tract damage.

Because exposure-response data were unavailable for all of the AEGL-specific exposure durations, temporal extrapolation was used in development of values for the AEGL-specific time periods. The concentration-exposure time relationship for many irritant and systemically acting vapors and gases may be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al. 1986). Data regarding AEGL-1-type ef

fects reported by Reed (1918), Reed et al. (1918), Guild et al. (1941), and Anderson (1942) indicate that, for exposure periods up to several hours, the concentration-exposure time relationship is a near-linear function (i.e., Haber's law where n=1 for $C^n \times t = k$) as shown by n values of 1.11 and 0.96. Therefore, the empirically derived, chemical-specific estimate of n =1 was used for derivation of the AEGL-1 and AEGL-2 values. However, in the absence of chemical-specific lethality data, time scaling for AEGL-3 values was performed using exponential extrapolation (n=3) for shorter time periods and linear extrapolation (n=1) for longer time periods. This procedure provides a somewhat more conservative (i.e., protective) estimate of the AEGL-3 values than would be obtained using the single n value based upon ocular irritation.

The AEGL-1 values were based on data from Anderson (1942), who found that an exposure concentration-time product of 12 mg·min/m^3 represented a threshold for conjunctival injection and minor discomfort with no functional decrement in human volunteers acutely exposed to sulfur mustard. An intraspecies uncertainty factor (UF) of 3 was applied for protection of potentially sensitive individuals. This adjustment was considered appropriate for acute exposures to chemicals whose mechanism of action primarily involves surface contact irritation of ocular tissue rather than systemic toxicity. Anderson (1942) noted that there was little variability in the ocular responses among the subjects in his study, thereby providing additional justification for the intraspecies UF of 3.

The AEGL-2 values for sulfur mustard were also developed using the data from Anderson (1942). Anderson reported that a Ct value of approximately 60 mg·min/m^3 represented the lowest concentration-time product for which ocular effects were sufficiently severe (visual impairment and irritation) as to be characterized as military casualties. The 60-mg·min/m^3 exposure was used as the basis for developing the AEGL-2 values because it represented an acute exposure that caused an effect severe enough to impair escape and, although not irreversible, would result in the potential for additional injury. Anderson (1942) characterized the 60-mg·min/m^3 Ct as representing the lower margin of the concentration-effect zone that would result in ineffective military performance (i.e., performance necessary to complete a mission) and that might require treatment for up to 1 week (wk). The ocular irritation and damage were also considered appropriate as a threshold estimate for AEGL-2 effects because the eyes are generally considered the most sensitive indicator of sulfur mustard exposure, and irritation would likely occur in the absence of vesication effects and severe

pulmonary effects. The fact that the AEGL-2 is based on human data precludes the use of an interspecies UF. A factor of 3 was applied for intraspecies variability (protection of sensitive populations). The factor was limited to 3 under the assumption that the primary mechanism of action of sulfur mustard involves a direct effect on the ocular surface and that the response will not vary greatly among individuals. Anderson also noted little variability in the ocular responses among the subjects in his study. A modifying factor of 3 was applied to accommodate potential onset of long-term ocular or respiratory effects. This was justified by the fact that there was no long-term follow-up reported by Anderson to confirm or deny the development of permanent ocular or respiratory tract damage. The total modifying factor adjustment was 10 (because the factors of 3 each represent a logarithmic mean [3.16] of 10, that is, 3.16×3.16=10).

For development of the AEGL-3, a 1-h exposure of mice at 21.2 mg/m^3 was used as an estimated lethality threshold (Kumar and Vijayaraghavan 1998). That value is also near the lower bound of the 95% confidence interval for the 1-h mouse LC_{50} of 42.5 mg/m^3 reported by Vijayaraghavan (1997). The intraspecies variability was limited to 3 because the lethality resulting from acute inhalation exposure to sulfur mustard appears to be a function of pulmonary damage resulting from direct contact of the agent with epithelial surfaces and would not likely exhibit an order-of-magnitude variability among individuals. A UF of 3 was also applied to account for possible interspecies variability in the lethal response to sulfur mustard. The resulting total UF adjustment was 10. The modifying factor of 3 used for AEGL-2 development to account for uncertainties regarding the latency and persistence of the irritant effects of low-level exposure to sulfur mustard was not applied for AEGL-3 because lethality of mice was assessed at 14 days (d) postexposure in a previous study by Vijayaraghavan (1997). Application of any additional UFs or modifying factors was not warranted because the AEGL-3 values are equivalent to exposures in humans that are known to produce only ocular and respiratory tract irritation.

The AEGL values for sulfur mustard are based on noncancer end points. Sulfur mustard is genotoxic and has induced carcinogenic responses in humans following single high-concentration exposure and following multiple exposures that were sufficient to produce adverse effects. Based on available sulfur mustard data and in the absence of clinical signs, carcinogenic responses in humans have not been observed following acute low-level or nonvesicating exposures. The human data summarizing cancer

incidences among individuals exposed to sulfur mustard is primarily that for wartime gas-factory workers and for military personnel who sustained injury following direct contact with "battlefield concentrations" of sulfur mustard liquid and/or vapor. A cancer risk assessment based on a geometric mean of inhalation slope factors developed using various data sets and procedures indicated an excess cancer risk of 1 in 10,000 (10^{-4}) may be associated with exposures similar to the AEGL-3 values. The use of excess-cancer-risk estimates in setting AEGL values is precluded by the uncertainties involved in assessing excess cancer risk following a single acute exposure of 8 h or less, the relatively small population exposed in an

TABLE 2–1 AEGL Values for Sulfur Mustard in Parts Per Million and Milligrams per Cubic Meter

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1[a]	0.06 ppm (0.40 mg/m^3)	0.02 ppm (0.13 mg/m^3)	0.01 ppm (0.067 mg/m^3)	0.003 ppm (0.017 mg/m^3)	0.001 ppm (0.008 mg/m^3)	Conjunctival injection and minor discomfort with no functional decrement in human volunteers (Anderson 1942)
AEGL-2[a]	0.09 ppm (0.60 mg/m^3)	0.03 ppm (0.20 mg/m^3)	0.02 ppm (0.10 mg/m^3)	0.004 ppm (0.025 mg/m^3)	0.002 ppm (0.013 mg/m^3)	Well-marked, generalized conjunctivitis, edema, photophobia, and eye irritation in human volunteers (Anderson 1942)
AEGL-3[a]	0.59 ppm (3.9 mg/m^3)	0.41 ppm (2.7 mg/m^3)	0.32 ppm (2.1 mg/m^3)	0.08 ppm (0.53 mg/m^3)	0.04 ppm (0.27 mg/m^3)	Lethality estimate in mice (Kumar and Vijayaraghavan 1998)

[a]AEGL-1 and AEGL-2 values, and the 4- and 8-h AEGL-3 values are at or below the odor threshold for sulfur mustard.

emergency release situation, and the potential risks associated with evacuations.

The AEGL-1 and AEGL-2 values are based on human exposure data and are considered to be defensible estimates for exposures representing thresholds for the respective AEGL effect levels. Ocular irritation, which forms the basis for AEGL-1 and AEGL-2 values, is the most sensitive response to sulfur mustard vapor. The AEGL-3 values provide Ct products (approximately 39–130 mg·min/m^3) that are known to cause moderate to severe ocular irritation and possible respiratory tract irritation in human subjects but no life-threatening health effects or death. It must be noted that all of the AEGL-1 and AEGL-2 values and the 4- and 8-h AEGL-3 values are at or below the odor threshold for sulfur mustard. In consequence, there is considered to be a finite amount of time to don protective equipment and safeguard critical target tissues such as the eyes and respiratory tract.

Although the overall database for acute inhalation exposure to sulfur mustard is not extensive, the AEGL values appear to be supported by the available data. Extrapolation to exposure durations of less than 10 min is not recommended in the absence of careful evaluation of existing exposure-response data and comparison of any derivative values with these data.

I. INTRODUCTION

Sulfur mustard (agent HD) is an alkylating chemical vesicant that affects any epithelial surface it comes in contact with. It has been developed and used as a warfare agent. The active component is bis(2-chloroethyl)sulfide (CAS Registry No. 505–60–2). Although the chemical is a liquid at ordinary ambient temperatures, its volatility results in rapid generation of vapors (see review by Watson and Griffin [1992]). Ambient temperature and humidity govern the degree of "casualty effect." Under hot and humid conditions, much lower mustard concentrations generate debilitating effects. Sulfur mustard has a garlic-like odor and, due to its low aqueous solubility, is persistent in the environment. Watson and Griffin (1992) have summarized information on the distribution of unitary chemical weapon stockpiles in the United States. Among various U.S. Army facilities, there were approximately 17,018.1 tons of sulfur mustard (agent HD) awaiting disposal in September 2001 (DA 2001). Pertinent physicochemical data for sulfur mustard are summarized in Table 2–2.

TABLE 2–2 Physicochemical Data for Sulfur Mustard

Synonyms	Agent HD; sulfur mustard; dichloroethyl sulfide; yperite; mustard gas; Bis(2-chloroethyl) sulfide; sulfide, Bis(2-chloroethyl); 1,1'-thiobis[2-chloroethane]; yellow cross; LOST	DA 1996; Budavari et al. 1989; Büscher 1932
Chemical formula	$C_4H_8Cl_2S$	Budavari et al. 1989
Molecular weight	159.08	DA 1996
CAS Registry No.	505–60–2	Budavari et al. 1989
Physical state	Oily liquid	DA 1996
Solubility	Sparingly soluble in water; soluble in organic solvents	DA 1996; Budavari et al. 1989
Vapor pressure	0.072 mm Hg at 20 °C 0.11 mm Hg at 25 °C	DA 1996
Density	5.4	DA 1996
Boiling/melting point	215–217 °C/13–14 °C	DA 1996; Budavari et al. 1989
Conversion factors in air	1 ppm=6.49 mg/m^3 1 mg/m^3=0.15 ppm	

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

Either inhalation or percutaneous exposure to sulfur mustard vapor can result in lethality, although inhalation exposure is the more sensitive route. Estimates of human LCt_{50} values for agent vapor inhalation are several times lower than the estimated human percutaneous LCt_{50} (Robinson 1967; DA 1974). This contention is supported by animal LCt_{50} data (Robinson 1967; DA 1974; Watson and Griffin 1992). Human lethality data are available only as estimates attained by extrapolation from animal data. The estimated human LCt_{50} values in use by the U.S. Army are 1,500 mg·min/m^3 and 10,000 mg·min/m^3 for inhalation and percutaneous vapor exposure, respectively (DA 1974; NRC 1997).

Although lacking quantitative exposure terms, Warthin and Weller (1919) provided qualitative clinical information regarding two fatalities resulting from sulfur mustard exposures during manufacture of the agent. Both men were wearing gas masks, so ocular involvement was inconsequential, but the exposure concentrations were high enough to result in severe skin burns. Within hours, both victims exhibited lesions about the lips and necrotic lesions in the mouth and nasopharyngeal region. By 7 to 8 d postexposure, there was evidence of more severe respiratory involvement, as demonstrated by moist rales and physical signs indicative of bronchopneumonia. One victim died 8 d after the accident, and the other died 4 wk after the exposure.

Between 1919 and 1923, site remediation and scrap metal recovery operations at a vast (25 square miles) "gas dump" at Breloh, Germany, near Munster in what is now Lower Saxony, resulted in numerous cases of occupational exposure to warfare agents either manufactured or captured by German forces during World War I (Büscher 1932). Thousands of tons of "gas" munitions as well as tank cars and storage buildings containing sulfur mustard and other chemical warfare agents were involved. Summary reports for the years 1920–1923 by the primary-care physician at the site document "two or three" fatalities among workmen who had received concentrated sulfur mustard vapor exposures to the skin, eyes, and respiratory tract in combination. In these cases, "death came very soon" (Büscher 1932). Büscher (1932) was not equipped to gather source term information for any of these fatal episodes.

Estimated lowest lethal doses of 150 mg/^{3} (10 min) and 70 mg/m^3 (30 min) have been reported (Back et al. 1972; Inada et al. 1978). However, those values are not based on definitive exposure values or controlled exposure conditions.

Available hospital records from World War I and sketchy casualty reports from the Iran-Iraq conflict indicate mortality rates of 1–3% from acute sulfur mustard exposure (Blewett 1986; Dunn 1986). Actual battlefield concentrations have not been reported but may well have been in excess of 1,500 mg/m^3 (Watson and Griffin 1992).

Human lethalities were reported by a number of European physicians asked to provide humanitarian treatment for gas casualties arising from the Iran-Iraq conflict. Eisenmenger et al. (1991) treated sulfur-mustard exposed Iranian patients in a German hospital; one patient admitted 5 d postexposure in a semiconscious state with serious exfoliative lesions died during treatment. Other Iranian soldiers exhibiting the characteristic burns,

edema, and damage to the respiratory tract associated with battlefield exposures to sulfur mustard died from various combinations of respiratory insufficiency and infection between 5 and 36 d postexposure (one on day 7, three on days 12–15, one on day 36; *N*=5) (D'Halluin and Roels 1984; Mandl and Frielinger 1984). Sulfur mustard agent is a known immunosuppressant (IOM 1993); however, no exposure terms for any of these wartime cases were available.

In an effort to establish updated toxicity estimates for humans, the U.S. Army Chemical Defense Equipment Process Action Team (Reutter and Wade 1994) developed a revised estimated LCt_{50} of 900 mg·min/m^3 for human inhalation exposure from an average of animal LCt_{50} data. The National Research Council Committee on Toxicology (Subcommittee on Toxicity Values for Selected Nerve and Vesicant Agents) concluded that the 900 mg·min/m^3 estimate was scientifically valid (NRC 1997) but cautioned that the estimate was developed with reference to healthy male military personnel and is *not* applicable to civilians.

2.2. Nonlethal Toxicity

Clinical presentation in humans following acute exposure to sulfur mustard vapor may involve dermal, ocular, and respiratory tract effects, all of which are preceded by a latency period dependent on the exposure concentration and exposure duration (Eisenmenger et al. 1991). Systemic effects (nausea, vomiting, abdominal pain, headache, weight loss, hematopoietic effects) may also occur as a result of gastrointestinal involvement or deep penetration dermal involvement (Büscher 1932). The eye appears to be the most frequently affected and most sensitive organ and also has one of the shortest latency periods (Warthin and Weller 1919; Papirmeister et al. 1991). Latency periods vary with changes in exposure parameters but tend to be several hours to days for dermal effects, 2–8 h for ocular effects, and several hours for upper respiratory tract effects (up to several days for progression to full severity respiratory tract involvement). Studies involving controlled exposure of human volunteers as well as studies on war casualties and occupational exposures are available; the latter provide clinical information but lack quantitative exposure data.

Controlled human clinical trials conducted by Büscher (1932) to better define treatment regimens were confined to "drop" tests of sulfur mustard on various skin sites with observations of the time course under differing

decontamination protocols. Inhalation exposures occurred to Breloh gas-dump workers as a consequence of munition explosions, inhalation of smoke plumes generated during primitive "bonfire" heat-cleaning of contaminated metal scrap, off-gassing of contaminated clothing in warm rooms, and the use of contaminated wood scraps as heating fuel in winter quarters. Büscher (1932) describes the clinical course of respiratory effects and their treatment but does not present dose-response data.

Reed (1918) conducted preliminary experiments in which he and another volunteer participated in exposure chamber experiments at a sulfur mustard concentration of 0.0012 mg/L (1.2 mg/m^3); mustard was generated as a spray in absolute ethanol for 45 min in a 10,000 L chamber. The subjects were clad in ordinary khaki uniforms, without blouses, and had no facial protection. A slight odor was initially detected but the olfactory response accommodated within 3 min for one subject and 8 min for the other. Slight irritation of the mucosa of the nose and nasopharyngeal regions occurred at 8 min and progressed in severity such that at 20 min one individual determined to be sensitive to HD on the basis of skin tests withdrew from the exposure chamber. At 25 min, the remaining subject experienced heavy eyelids and "huskiness" of the voice but no coughing or sneezing. At 3 h after the 45-min exposure and 6 h after the 20-min exposure a sudden and severe conjunctivitis developed that was accompanied by photophobia and blepharospasm. By 12 h postexposure, vision was severely impaired, and severe pain and rhinitis were experienced for 30 h. These effects were somewhat less severe in the subject originally classified as more sensitive. Conjunctival injection did not resolve for over a month. At 3 d postexposure, intense pruritus and erythema developed over the neck, shoulders, upper arms, and trunk. It began abating after 7 d. Ocular hypersensitivity and exercise-induced dermal wheals occurred for weeks after the exposure.

Reed (1918) conducted additional experiments using lower sulfur mustard concentrations. In those experiments, one to six volunteers were exposed at various low concentrations of sulfur mustard (0.0001–0.0043 mg/L, nominal; equivalent to 0.1–4.3 mg/m^3) for time periods of 5 to 45 min. The exposure atmospheres were generated by slowly spraying sulfur mustard in absolute alcohol and continually mixing the air with an electric fan. Subsequent investigations revealed that the actual exposure concentrations were ≤60–70% of nominal, although Reed (1918) freely admitted that "it is impossible to state what the actual concentration was" due to analytical limitations of the time. It is assumed from context that the volunteers

were clothed similarly to those in initial trials (e.g., khaki uniforms without blouses) and wore no facial protection during the period of exposure. Of the 22 men participating in this series (see Table 2–3), a majority had been exposed to sulfur mustard before, and 12 had sustained "one or more burns" either experimentally or accidentally (Reed 1918). The most prominent effect of the controlled atmospheric exposures was ocular irritation (conjunctival injection, conjunctivitis, photophobia), which varied among individuals depending on exposure concentration and duration. The results of these experiments are summarized in Table 2–3.

Reed et al. (1918) also conducted experiments that utilized improved methods (e.g., hydrogen ion method) for measurement of exposure concentrations. To minimize hydrolysis, the HD was delivered in absolute alcohol.

Walker et al. (1928) reported that of seven men exposed to sulfur mustard at 0.001 mg/L (1 mg/m^3) for 5–45 min, four showed conjunctivitis and two exhibited skin burns. It was also reported that of 17 men exposed at 0.0005 mg/L (0.5 mg/m^3) for 10–45 min, six exhibited conjunctivitis, one had a skin burn, and that three of 13 men exposed for 10–30 min at 0.0001 mg/L (0.1 mg/m^3) showed slight but distinct conjunctivitis.

Guild et al. (1941) conducted experiments using human volunteers exposed to sulfur mustard at varying acute exposure regimens. The sulfur mustard vapor was generated by heat volatilization in a 100-m^3 exposure chamber. The subjects were male soldiers and officers and one civilian who had not had previous exposure to sulfur mustard. All subjects wore paint or "dope" spray respirators "to protect the lungs" (Guild et al. 1941). For each of the tests, two to six individuals were exposed. Guild et al. concluded that Ct is constant for ocular effects for exposure periods of 2 min to 20 h and for sulfur mustard concentrations of 0.07–65 mg/m^3. Based on the results of the experiments, it was reported that exposure at Ct values <70 mg·min/m^3 would result in mild conjunctival responses that would not be indicative of a casualty (defined by the authors as temporary loss of vision); Ct values at 70–100 mg·min/m^3 would produce some casualties; and Ct values at >100 mg·min/m^3 would be expected to produce disabling ocular effects for several days. In the military context of this study, Guild et al. (1941) defined "disablement" as "injury sufficient to prevent troops from taking an active part in operations for 1–2 weeks." Because the subjects wore respiratory protection, effects on the respiratory tract could not be determined and were not reported.

TABLE 2-3 Effects of Acute Exposure to Sulfur Mustard (Agent HD) in Human Volunteers

Nominal Concentration (mg/m^3)	Exposure Duration (min)	Number of Subjects	Results
0.1	10	6	No detectable effect
0.1	15	2	One of two subjects exhibited slight conjunctival injection
0.1	30	5	One of five showed marked bilateral conjunctival injection; one of five showed slight conjunctival injection
0.5	10	5	Two of five exhibited conjunctival injection
0.5	15	3	One of three exhibited slight conjunctival injection
0.5	30	8	One of eight exhibited conjunctivitis and experienced rhinitis; one of eight exhibited severe conjunctivitis, marked skin burn; one of eight exhibited marked conjunctivitis, slight facial burn
0.5	45	1	No effect
1.0	5	1	Marked conjunctivitis, photophobia, rhinitis, laryngitis, pulmonary congestion
1.0	10	2	One of two exhibited slight conjunctivitis
1.0	15	2	No effect
1.0	20	1	Exhibited severe conjunctivitis, severe skin burns
1.0	45	1	Very severe conjunctivitis, photophobia, skin burns, mucosal exfoliation in nasopharynx
2.6	5	1	No effect
4.3	10	1	Marked conjunctivitis, no pain

Note: Unprotected face assumed from study context.
Source: Reed 1918.

In a study reported by Anderson (1942) and performed as a follow-up to the Guild et al. (1941) recommendation to replicate the earlier Guild experimental design under tropical conditions, three to four human volunteers were exposed to each of several concentration-time regimens of agent HD "under Indian hot weather conditions." Sulfur mustard vapor was generated by heat volatilization in a 50-m^3 exposure chamber; mixing was accomplished by use of an electric fan in the chamber. Subjects included both British and Indian troops without respiratory protection who wore tropical service dress of drill shorts and open-necked cotton shirts. To minimize off-gassing exposure, subjects bathed and dressed in clean clothing upon completion of each experiment. Eyes of each subject were examined prior to the first experimental exposure; the author noted that a certain degree of fine conjunctival injection was a normal baseline condition for a large proportion of persons living in India at that time. Allowance was thus made for this baseline condition in assessing postexposure effects to sulfur mustard vapor. Effects on the respiratory tract were not reported.

Anderson (1942) determined HD concentrations by use of the gold-benzidine method and performed analysis in a "Spekker photoelectric absorptiometer." In an analysis of the data and cross-comparison with the temperate-zone results of Guild et al. (1941), Anderson determined that comparable eye effects of a particular degree of severity are usually produced at a lower Ct under tropical conditions. An exposure concentration-time product of 30 mg·min/m^3 represented the upper range for mild effects with no disability (conjunctival injection and minor discomfort with no functional decrement). Ct products slightly higher than that (e.g., 34–38.1 mg·min/m^3) were, however, also without appreciable casualty effects. A concentration-time product of 12 mg·min/m^3 was noted by Anderson (1942) as representing the limit for ocular effects as characterized by conjunctival injection in the complete absence of irritation. Ct values of 60–75 mg·min/m^3 were considered a danger zone for widespread conjunctivitis frequently accompanied by chemosis, photophobia, and irritation. At Ct values of 75–90 mg·min/m^3, more severe ocular effects would be expected, to the extent that several weeks of treatment would be necessary in a high proportion of subjects so exposed. At Ct values ≥100 mg·min/m^3, a 100% casualty rate (as determined by militarily disabling ocular effects) would be expected. The results of these experiments are summarized in Table 2–4.

Please note that the longest reported period of follow-up in the Anderson (1942) study was 36 d postexposure for a case requiring infirmary treatment and exhibiting conjunctivitis, photophobia, and injection with

TABLE 2-4 Effects of Acute Exposure to Sulfur Mustard (Agent HD) in Human Volunteers

Mean Concentration (mg/m^3)	Exposure Duration (min)	Number of Subjects	Cumulative exposure (Ct) ($mg \cdot min/m^3$)	Results
6.25	2	4	12.5	Three of four—band of fine injection across exposed bulbar conjuntiva; one of four—trace angular conjunctivitis; all noncasualties
7.0	3.3	4	23.1	Three of four—obvious band of injection across exposed bulbar conjuntiva; one of four—angular conjunctivitis; all noncasualties
10.0	2.75	3	27.5	Two of three—mild injection band over exposed sclera; one of three—band of injection with slight discomfort; all noncasualties
6.8	5	3	34.0	Three of three—well-marked injection of conjunctivae; slight edema in oneof three; all complaining of eye soreness; injection visible in one of three at 14 d postexposure; all noncasualties
12.7	3	3	38.1	Three of three—band of conjunctival injection over exposed sclera; no discomfort; all noncasualties
12.6	3.3	3	41.8	Three of three—effects slightly more marked than in previous experiment; mild discomfort in one of three; all noncasualties

11.0	4	3	44.0	Three of three—moderate injection of exposed bulbar conjunctiva and lower lids (to a lesser degree); one of three—slight edema; one of three—complained of sore eyes in first 24 h; all noncasualties
7.6	6	4	45.6	Three of four—widespread conjunctivitis involving lids and bulb; one of four—exhibiting trace chemosis; one of four—slight photophobia on days 2 and 3; one of four—moderate band of injection; all complaining of discomfort
13.0	3.75	3	48.8	Three of three—widespread moderate injection of conjunctiva; one of three—slight discomfort; one of three—transient edema; all noncasualties
10.5	4.75	3	49.8	Three of three—well-marked injection of lids and exposed conjunctiva; two of three—discomfort; all noncasualties
2.5	20	3	50.0	Three of three—band of moderate injection over exposed part of sclera; two of three—slight soreness
10.6	5	2	53.0	Two of two—widely generalized conjunctival injection visible after 14 d; one of three—complaining of sore eyes; all noncasualties
15.6	3.5	1	54.6	Band of injection across exposed part of sclera; slight conjunctival injection; soreness in one eye; noncasualty.

Mean Concentration (mg/m^3)	Exposure Duration (min)	Number of Subjects	Cumulative exposure (Ct) ($mg \cdot min/m^3$)	Results
5.8	9.5	4	55.1	One of four—casualty; wide and intense redness over entire conjunctiva, slight photophobia, moderate chemosis and blepharospasm; three of four—just short of casualty with widespread conjunctival injection, slight edema, and mild photophobia in first 24 h, sore eyes for 2-3 d
14.0	4.0	3	56.0	Three of three—well-marked and widespread conjunctival injection, discomfort; all noncasualties
1.7	33	3	56.1	Three of three—fine injection band over exposed sclera; all noncasualties
2.9	20	3	58.0	Three of three—moderate and generalized conjunctival congestion; one of three—mild discomfort; all noncasualties
4.5	13.5	3	60.7	Three of three—band of moderate injection over exposed sclera; one of three—reported headache on day 1 and later developed generalized urticaria; all non-eye casualties

13.7	4.75	3	65.0	Two of three—widespread conjunctival injection, slight edema and mild discomfort; one of three—severe injection of conjunctiva, well developed edema, very near casualty, severe urticarial reaction first day post-exposure, positive reaction to 1:25,000 sulfur mustard after 1 mo
5	14	3	70	Two of three—well marked and generalized conjunctivitis with edema, photophobia, lacrimation and blepharospasm; sore eyes and frontal headache, casualties up to 1 wk; one of three—intense congestion of entire conjunctiva, lacrimation, chemosis and photophobia
15.6	4.5	2	70.2	One of two—injection of lids, well-marked band of injection across exposed sclera, soreness up to day 3, noncasualty; one of two—severe conjunctival injection, slight hazing of cornea with photophobia and soreness up to day 3 postexposure, lacrimation and slight interference with vision, casualty requiring 4-5 d treatment
4.7	15	3	70.5	Three of three—lids injected, well-marked and generalized conjunctivitis with edema, photophobia and eye soreness; one of three—headache; all near casualties requiring 3-5 d treatment

Note: No respiratory protection was worn during exposure periods.
Source: Anderson 1942.

corneal injury. By discharge on day 36, both eyes were reported "normal." It is observed that, during the 1940s, it was common practice to employ minimal long-term medical follow-up in studies of military personnel experimentally exposed to chemical warfare agents (IOM 1993). Short-term casualty effects were the primary focus of military investigators at the time.

Following a review and evaluation of all available data, an ECt_{50} of 100 mg·min/m^3 for severe ocular effects (for soldiers) was determined by Reutter and Wade (1994) and the NRC (1997). The estimate was based on an assumed exposure duration of 2 to 10 min and an effect severity consistent with that which would necessitate removal of soldiers from the battlefield. The assessment also affirmed that the eye is a sufficiently sensitive organ on which to base exposure estimates.

The percutaneous absorption of sulfur mustard vapor in human skin was studied by Nagy et al. (1946) to understand more fully the relationship between penetration rate and severity of toxicity. Using a carefully designed and tested technique and human volunteers, Nagy et al. determined the penetration rate of sulfur mustard for human skin. The application times studied using human skin were 3, 6, and 10-min exposures. A saturated atmosphere (under an application cup) of sulfur mustard was applied to a 1.3-cm^2 area of the flexor aspect of the forearm; lesions (pinhead vesicles, erythema, vesication) were evaluated at 48 h after application. Quantitation of agent that penetrated the skin was determined by comparing the quantity of HD vapor in the application cup before and after a given time interval. It was found that an increase in temperature (from 21–23 °C to 30–31 ° C) produced an increase in the penetration rate from 1.4 μg/cm^2/min to 2.7 μg/cm^2/ min.

Moore and Rockman (1950) studied variability in hypersensitivity reactions to sulfur mustard using human volunteers. A single drop (4.5± 0.22 mm^3) of various dilutions of purified sulfur mustard (1:500 to 1:8,000 in petroleum ether) was applied to each subject's volar forearm. The test area was examined at 24, 48, and 72 h, and a description of the reaction was recorded. About 25% of those given two exposures to sulfur mustard with a week exhibited a flare response at the first site even when the second application was at a different site (e.g., opposite arm). Although a conversion of this exposure regimen to an equivalent air concentration was not feasible, the results of the study provide evidence of possible dermal sensitization to sulfur mustard dermal exposure. Similar findings are reported in Büscher (1932), Sulzberger et al. (1945), and IOM (1993).

Warm, moist anatomical areas such as the axillae and groin are especially susceptible to sulfur mustard vapor injury (IOM 1993).

Eisenmenger et al. (1991) reported clinical and morphologic findings from 11 Iranian patients exposed to sulfur mustard during the Iran-Iraq conflict and treated in a German hospital. Quantitative exposure data are lacking for these case reports, but the information provides a clinical picture of the progression of sulfur mustard lesions. Upon admittance to the hospital (4–6 d or 17 d after exposure), all patients exhibited conjunctivitis and some also exhibited erosions and slight corneal opacity and reddened, blistered skin. The severity of respiratory tract involvement tended to be concentration-dependent, with only upper respiratory tract involvement at lower concentrations. The most serious respiratory effects were observed at 14 d postexposure. One patient admitted in a semiconscious state with serious exfoliative lesions observed at 5 d postexposure died, and several others likely would have died without medical intervention. No follow-up study was performed on these patients. Although lacking quantitative data useful for developing AEGL values, this clinical report provides qualitative information regarding human exposure to sulfur mustard and indicates that effects observed in humans are similar to those observed in animals.

Odor thresholds of 1 mg·min/m^3 (Bloom et al. 1944), 0.15 mg/m^3 (Ruth 1986), and 0.6 mg/m^3 (Dudley and Wells 1938; Bowden 1943; Fuhr and Krakow 1945) have been reported.

2.2.1. Epidemiologic Studies

Emad and Rezaian (1997) conducted a cross-sectional clinical study of late pulmonary sequelae exhibited by 197 Iranian military veterans 10 y after receiving a single, high-concentration sulfur mustard exposure in 1986 during the Iran-Iraq conflict. The control group consisted of 86 nonexposed veterans. In 1986, exposure to sulfur mustard had been initially confirmed at hospital admission by urine and vesicular fluid analysis (by the method of Heyndrickx et al. [1984]) and by presentation with respiratory symptoms that included rhinorrhea, sore throat, hoarseness, cough, chest tightness, and dyspnea. Participants were screened for asthma and prior exposures to environmental agents known to cause interstitial lung disease or extrinsic allergic alveolitis. In addition, participants were not allowed to have had jobs that might create interference with the study (e.g., woodworking, milling, welding, farming, sculpturing, painting, fire fighting, baking) since 1986. The incidences of asthma (10.65%), chronic bronchitis (58.88%), bronchiectasis (8.62%), airway narrowing due to scar or granulation tissue (9.64%), and pulmonary fibrosis (12.18%) in the sulfur

mustard exposed group were all greater than those found in the referent group (0% in all categories except for one case of bronchitis [1%]). The investigators concluded that exposure to clinically significant sulfur mustard concentrations created greater potential for development of chronic destructive pulmonary sequelae. The authors further concluded that the relatively low incidence of pulmonary fibrosis resulted from the fact that the largest proportion of mustard agent was absorbed in the upper airways rather than in the alveoli. No bronchial carcinoma or lung malignancy has been observed to date in this group of veterans (Emad and Rezaian 1997).

2.3. Neurotoxicity

There are no data currently available regarding potential neurotoxic effects of inhaled sulfur mustard in humans.

2.4. Developmental and Reproductive Toxicity

There are no data currently available regarding potential developmental and reproductive toxicity of inhaled sulfur mustard in humans.

2.5. Genotoxicity

The International Agency for Research on Cancer (IARC) (1975, 1982, 1987a,b), Fox and Scott (1980), ATSDR (1992), Papirmeister et al. (1991), and Watson and Griffin (1992) summarized the evidence concerning genotoxicity of sulfur mustard. Because sulfur mustard is a potent DNA alkylating agent, genotoxic effects occur through cross-link formation, inhibition of DNA synthesis and repair, point mutations due to replication or repair errors, chromosome breaks, and chromatid aberrations. Some of those conditions have been observed in humans following exposure to sulfur mustard, others have occurred in various test systems including bacteria, yeast, insects, and mammalian cell cultures.

Retrospective studies have been conducted on Japanese workers who were employed at a chemical agent manufacturing plant from 1929 to 1945. Although sulfur mustard was the main product of the facility, lewisite, diphenylarsine, hydrocyanic acid, phosgene, and chloroacetophenone were

also produced there (Inada et al. 1978), and it is not known to what degree those other chemicals contributed to the observed effects. In one study of the workers, Yanagida et al. (1988) found that the frequency of mutations to hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT) deficiency in 28 exposed individuals was significantly elevated when compared with two control groups matched for age and smoking status. One control group consisted of healthy men and the other of individuals with bronchitis. The data also showed that the mutations were significantly more frequent in workers who had longer exposures. A chromosome study of 16 former workers of this same factory indicated a significantly higher incidence of sister chromatid exchanges (SCE) in peripheral lymphocytes when compared with a control group ($p<0.03$) (Shakil et al. 1993). Two individuals with chronic myelocytic leukemia had an almost 3-fold higher SCE rate than controls and also a high (12.1%) incidence of chromosome abnormalities (Shakil et al. 1993). In an evaluation of the p53 mutations found in lung tumors of these workers, Takeshima et al. (1994) found that the mutations were similar to those in lung tumors of tobacco smokers (the factory workers were also tobacco smokers); however, the prominence of G:C to A:T transitions and the occurrence of double mutations in two of 12 cases suggested that exposures in the chemical agent manufacturing plant contributed to the development of the lung cancers.

Yamakido et al. (1985) studied the potential genotoxicity of sulfur mustard in children of workers previously exposed at a Japanese poison gas factory. The study utilized general health exams in conjunction with one-dimensional electrophoretic analysis of blood protein variants to identify gene mutations. Although variants were detected, the investigators considered the results inconclusive as to the potential genotoxicity of sulfur mustard in humans because of the small size of the population sampled.

Wulf et al. (1985) reported significant ($p<0.001$) increases in sister chromatid exchanges in lymphocytes of 11 fisherman who had accidentally been exposed to sulfur mustard in sufficiently high concentrations to cause signs of acute toxicity. The fishermen received contact exposure to sulfur mustard from nets deployed in areas where World War II-era munitions had been dumped at sea.

Cytometric analysis of DNA damage was shown for cultured human epithelial cells exposed to sulfur mustard (Emison and Smith 1997). The cell cycle was found to be blocked at the G1-S interface at concentrations equivalent to an in vivo vesicating dose (>100 μM) and is blocked in the G2 phase at concentrations below an equivalent vesicating concentration.

At concentrations of 3 μM, the cell cycle was initially blocked at G2/M, but the cells recovered normal cell cycle progression. Quantitation of DNA strand breaks was possible at concentrations equivalent to both vesicating and nonvesicating exposures.

2.6. Carcinogenicity

Studies evaluating workers occupationally exposed to sulfur mustard indicate elevated risks of respiratory tract and skin tumors after long-term exposure. Genotoxicity and animal carcinogenicity data as well as information characterizing the alkylating properties of sulfur mustard provide supporting evidence for the carcinogenicity of sulfur mustard in humans. This work has been summarized in USACHPPM (2000).

IARC classified sulfur mustard as a Group-1 compound (carcinogenic to humans) (IARC 1987), and the National Toxicological Program (NTP) first categorized sulfur mustard gas (or mustard gas) as a substance "known to be a human carcinogen" in its *First Annual Report on Carcinogens, 1980*. Mustard gas is still listed in the same category in the *Ninth Report On Carcinogens, 2000* (DHHS 2000). The State of Maryland also considers mustard gas a "known human carcinogen" (a Class I.A. Toxic Air Pollutant as defined by the Code of Maryland Regulations, CMR Title 26 Subtitle 11, amended).

IARC (1975), Waters et al. (1983), Watson et al. (1989), and IOM (1993) summarized the epidemiological evidence concerning the potential carcinogenicity of sulfur mustard in humans. Those data are primarily from studies of soldiers exposed during World War I and from studies of workers at chemical warfare agent manufacturing facilities.

Individual case studies of World War I veterans include Case and Lea (1955) and Beebe (1960). Case and Lea (1955) reported that the mortality ratio (2.07) of 1,267 World War I United Kingdom veterans indicated a highly significant elevated risk for respiratory tract neoplasms ($p<0.01$). A similar tumor incidence rate and mortality ratio (2.01) were found in a population of veterans who had never been exposed to mustard gas but were suffering from bronchitis. Case and Lea (1955) concluded that the evidence did not support the view that sulfur mustard was a direct carcinogen.

Beebe (1960) evaluated the occurrence of respiratory tract cancers among a group of 2,718 American soldiers exposed to sulfur mustard dur

ing World War I and found that the ratio of observed to expected cases was 1.47 (based on U.S. mortality rates) compared with 1.15 for wounded soldiers not exposed to sulfur mustard, and 0.81 for soldiers who had pneumonia but had not been exposed to mustard gas. Norman (1975) evaluated the same group of soldiers after a 10-y follow-up period (the study completed in 1965) and found that the exposed men had a 40% excess of lung cancer mortality, with an estimated relative risk of 1.3 (95% confidence limits of 0.9–1.9), compared with a control group consisting of wounded soldiers who were not exposured to mustard gas. The latency period was estimated at 22–37 y. Norman (1975) further concluded that there was no evidence in this limited data set that sulfur mustard exposure and cigarette smoking had a synergistic effect on lung cancer mortality.

Retrospective studies of Japanese workers who were employed at a chemical warfare agent manufacturing plant from 1929 to 1945 have revealed that those individuals have an increased risk of developing respiratory tract cancers (see Yamakido et al. [1996] for the most recent review). Although sulfur mustard was the main product of the facility, lewisite, diphenylarsine, hydrocyanic acid, phosgene, and chloroacetophenone were also produced (Inada et al. 1978). The concentration of sulfur mustard in the workplace was estimated to be as high as 50–70 mg/m^3 (Nakamura 1956), and workers frequently exhibited signs of sulfur mustard toxicity during the period of agent manufacture; those signs included acute conjunctivitis, acute rhinitis, acute bronchitis, and acute dermatitis with blister formation. Studies completed in the 1950s documented individual cases of bronchial and laryngeal carcinoma in this population of workers (Yamada et al. 1953, 1957; Yamada 1963) and an elevated incidence of deaths due to cancers of the respiratory tract and oropharynx (16.3% versus 0.4% in nonexposed inhabitants of the same geographic area). Elevated mortality rates among the former factory workers due to respiratory tract cancer was later confirmed by Wada et al. (1968). Neoplasms occurred in the tongue, pharynx, sphenoidal sinus, larynx, trachea, and bronchi; only one occurred peripherally in the lung. The median length of employment at the chemical warfare agent manufacturing facility was 7.4 y, and the median interval between first employment and death from cancer of the respiratory tract was 24.4 y (Wada et al. 1968).

Additional studies of this population of workers were conducted by Nishimoto et al. (1988) who incorporated histopathological and mortality data gathered between 1952 and 1986. For 1,632 of the workers, the overall standardized mortality ratio (SMR) for respiratory tract tumors was 3.9

(70 observed versus 17.8 expected, $p<0.001$, based on data for the Japanese male population) and the overall SMR for all malignant tumors was 1.2 (173 observed versus 142 expected, $p<0.01$). Age-adjusted SMRs for total malignancies, respiratory tract tumors, and gastrointestinal tract tumors showed significantly higher SMRs for the age-groups from 40 to 80 y.

Nishimoto et al. (1988) also found that the SMR was about 2.7 for individuals who had worked at the factory 0.5 to 5 y but was 7.17 for individuals who had been employed for more than 5 y. The SMR was not significantly elevated for individuals who had worked at the factory for 7 months (mo) or less.

Data on this same group of workers followed up to 1992 and has been summarized by Yamakido et al. (1996). The results do not differ substantially from those of Nishimoto et al. (1983, 1988).

Of 488 former workers who received dermatological examination, 115 had abnormal pigmentation and 22 had skin tumors, of which 8 were cases of Bowen's disease (intraepidermal squamous cell carcinoma) (Inada et al. 1978). Hyperkeratotic skin lesions, such as Bowen's disease, basal cell carcinomas, and hyperkeratotic papular eruptions, were present in 14 of 109 cases engaged only in sulfur mustard production and in 1 of 16 cases engaged only in lewisite production. No abnormalities were observed in 77 former factory workers who had no exposure to chemical agents (Inada et al. 1978). It was also observed that the longer an individual had been exposed to sulfur mustard, the more marked the skin lesions tended to become (Inada et al. 1978).

The studies of Yamakido et al. (1996), Nishimoto et al. (1988), Yamada (1974) and Inada et al. (1978) provide strong evidence for a causal link between chemical agent exposure and cancer of the respiratory tract; however, because the workers were potentially exposed to lewisite as well, it is not possible to state conclusively that the cancers were due solely to sulfur mustard. Furthermore, it should be noted that several possible confounding factors, such as tobacco smoking habits, preexisting health conditions, and postexposure occupational histories of the workers, were not evaluated. In addition, the SMR may not provide a good estimate of cancer risk, because it does not take into account the impact of medical intervention and socioeconomic factors that can affect survival rates.

Weiss and Weiss (1975) conducted studies evaluating the health of 271 workers employed for varying lengths of time between 1935 and 1945 at a munitions depot where the production, testing, and destruction of sulfur

and nitrogen mustard (as well as bromoacetone, phosgene, chloropicrin, and organic arsenicals) had occurred. Ninety percent of the group had chronic health problems, and 114 had died by the end of 1974. Thirty-five percent died from cancer, of which 38% were bronchial cancers. The total number of deaths from cancer was significant ($p<0.01$), and the number of bronchial cancers was also significant (11 observed versus 5 expected for the population of the geographic region where the facility was located). The number of cancers of the gastrointestinal tract was 35% greater than expected. The average tumor induction time was 21.6 y. IARC (1975) noted that the study was limited to workers with available medical records, which "raises the possibility that the proportion with cancer may have been inflated, since medical records or autopsy records would more likely have been preserved for workers with cancer." Furthermore, IARC (1975) does not mention whether Weiss and Weiss (1975) accounted for smoking habits and other confounding factors.

According to Klehr (1984), German workers involved in the dismantling of a sulfur mustard facility developed multiple skin lesions including basal cell carcinomas, Bowen's disease, Bowen's carcinomas, and carcinoma spinocellulare. The incidence rate for all tumors (including skin tumors) was 34% in 53 workers evaluated.

Manning et al. (1981) evaluated the incidence of cancer among former workers of a British sulfur mustard manufacturing facility (1939–1945). As of 1974, the number of deaths from all neoplasms combined (45) was slightly greater than that expected from national death rates, but the increase was not statistically significant. In follow-up investigations of this cohort, Easton et al. (1988) evaluated the mortality records of 3,354 workers and found greater numbers of cancer deaths when compared with national mortality rates. Significant increases were observed in deaths from cancer of the larynx, pharynx, and all other buccal cavity and upper respiratory sites combined. There were also elevated numbers of deaths from lung cancer compared with those expected ($p<0.001$). It was reported that the risks of developing cancer of the lung and pharynx were significantly related to the duration of employment. Significant excess mortality was also observed for cancers of the esophagus and stomach, but there was no correlation with the time since first exposure or the duration of exposure.

Manning et al. (1981) concluded that it was very likely that the observed cancers of the pharynx, larynx, and other upper respiratory sites were due to exposure to sulfur mustard because the excesses were too large to be accounted for by confounding factors (the effects of smoking, how

ever, were not evaluated). They increased with increasing duration of employment and were limited to the period >10 y after first employment. Evidence for a causal relationship between sulfur mustard exposure and other cancers, including lung cancer, was not considered to be as strong.

Although a large number of American military personnel were exposed to sulfur mustard in chamber and field tests conducted during World War II, the morbidity and mortality records of that cohort have not been adequately evaluated to document long-term health risks (IOM 1993).

Evaluations of available human and lab animal data sets have resulted in numerous estimates of a slope factor for sulfur mustard (Bakshi et al. 2000; McNamara et al. 1975; NRC 1999; Rosenblatt 1987; USACHPPM, 2000; USEPA 1991; Watson et al. 1989). The range of inhalation unit risk factors documented in this literature is 9.0×10^{-2} to 7.4×10^{-4} per $\mu g/m^3$ (geometric mean of 4.1×10^{-3} per $\mu g/m^3$) (USACHPPM 2000).

2.7. Summary

Human data regarding nonlethal effects of sulfur mustard are available from studies using volunteer subjects. Qualitative descriptions of the clinical presentation of injury following exposure to sulfur mustard vapor are also available for war casualties and occupational exposures. Lethality data for humans are not available, but LCt_{50} values have been estimated based on extrapolation from animal data.

The available data suggest that the location and severity of damage resulting from exposure to sulfur mustard are concentration-dependent and a function of the highly reactive nature of sulfur mustard (Papirmeister et al. 1991). Ocular surfaces appear to be a sensitive, rapidly responding target (Reed 1918; Reed et al. 1918; Anderson 1942). At low exposures, sulfur-mustard-induced injury appears to be limited to the upper respiratory tract (Eisenmenger et al. 1991) and eyes (Reed 1918; Reed et al. 1918; Anderson 1942). Anderson (1942) considered Ct values of 60–75 $mg\cdot min/m^3$ representative of exposures that would result in conjunctivitis, photophobia, and ocular irritation, while Ct values of 75–90 $mg\cdot min/m^3$ would cause a high proportion of casualties, defined by more severe ocular damage requiring several weeks of treatment. At higher concentrations, the pulmonary regions are also affected (Eisenmenger et al. 1991). For all targets, there is a latency period between initial exposure and development of effects. The eyes and respiratory tract appear to have the shortest la

tency period; usually a matter of hours depending on the severity of exposure.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Rats

Fuhr and Krakow (1945) reported 2-, 30-, and 60-min LCt_{50} values of 1,512, 990, and 840 mg·min/m^3, respectively, for rats. However, data are unavailable for verifying the values or the analytical techniques utilized in their development.

3.1.2 Mice

Fuhr and Krakow (1945) also reported 2-, 30-, and 60-min LCt_{50} values of 4,140, 1,320, and 860 mg·min/m^3, respectively, for mice. As is the case for rats, data are unavailable for verification.

In a head-only inhalation study, groups of four adult female Swiss mice (24–26 g) were exposed to sulfur mustard (>99% purity) at concentrations of 8.5, 16.9, 21.3, 26.8, 42.3, or 84.7 mg/m^3 for 60 min (Vijayaraghavan 1997). A group of mice exposed to filtered air for 60 min served as controls, and mice exposed to acetone vapor served as vehicle controls. Respiratory patterns of the mice were monitored for 7 d, and the animals were observed for up to 14 d postexposure. Sulfur mustard vapor was generated using a known quantity of sulfur mustard diluted with acetone and pumped into a compressed air nebulizer. Pressure in the nebulizer was adjusted for complete evaporation of the acetone diluent. A constant air flow of 20 L/min was maintained in the 50 cm×10 cm exposure chamber (constructed of PTFE). The chamber air was sampled at a rate of 50 mL/min for 5 min and analyzed by gas chromatography (flame ionization detector). The primary focus of the study was assessment of changes in respiratory patterns, and to that end, an RD_{50} of 27.4 mg/m^3 (RD_{50} is the exposure concentration necessary to evoke a 50% decrease in respiratory rate) was determined along with other effects on respiration described in Section 3.2.3. The study author noted that mice started dying 6 d after exposure to

"higher concentrations," and the author provided a 60-min LC_{50} of 42.5 mg/m^3. No exposure-response data or other details regarding lethality were provided except the confidence interval for the LC_{50}, which was very large (13.5–133.4 mg/m^3) because sensory irritation and decreased respiratory frequency of mice in the higher exposure groups affected the actual intake and absorption of the sulfur mustard (most likely only for the latter half of the exposure period, because the mice did not exhibit notable decrement in respiratory function during the first 15–20 min of exposure).

Kumar and Vijayaraghavan (1998) provided additional information regarding the lethal response of mice exposed to sulfur mustard. Groups of 30 female albino mice were exposed (head only) for 1 h to sulfur mustard at concentrations of 21.2, 42.3, or 84.6 mg/m^3 (equivalent to 0.5, 1.0, and 2.0 LC_{50}) and sacrificed at 6, 24, or 48 h or 7 d after exposure. Three groups of 10 mice were exposed at each concentration. The exposure system was as previously described by Vijayaraghavan (1997). No mice died during the exposure and none of the mice in the lowest exposure group died prior to scheduled termination. Within 7 d, however, five mice from the 42.3 mg/m^3 group and eight mice from the 84.6 mg/m^3 group died. It was not stated when the mice expired, and because groups of mice were terminated at three time points prior to 7 d postexposure, it was not possible to determine the overall 7-d mortality rate.

3.1.3. Guinea pigs

Langenberg et al. (1998) provided data on the lethality of inhaled sulfur mustard in guinea pigs. In this study, which examined both the toxicity and toxicokinetics of sulfur mustard, male hairless guinea pigs (eight per group) were exposed to sulfur mustard by nose-only inhalation or by percutaneous exposure to vapors. The investigators reported the 96-h LCt_{50} for 5-min exposure to be 800 mg·min/m^3 (95% confidence interval of 700–920 mg·min/m^3). No percutaneous exposure lethality values were provided because of difficulties with the exposure system when exposing the guinea pigs to concentrations consistent with percutaneous LCt_{50} values (10,000 mg·min/m^3) previously reported in the literature. The vapor-generating system and exposure system were modified from those used in nerve agent studies. Modifications included replacement of portions of the chamber so that they would be inert to sulfur mustard and an increase in chamber temperature (thermostat controlled at 25–30 °C) to accommodate the lower vapor pressure of sulfur mustard.

Rosenblatt et al. (1987) cite an LCt_{50} value at 900 mg·min/m^3 for the rabbit for a 10-min exposure duration. However, data were unavailable to verify that value or the analytical techniques utilized in its development.

3.2. Nonlethal Toxicity

3.2.1. Dogs

McNamara et al. (1975) conducted long-term inhalation studies of sulfur mustard in several species, including dogs. In those experiments groups of dogs (gender and strain not specified) were exposed continuously at 0.001 mg of sulfur mustard per cubic meter or discontinuously (6.5 h/d, 5 d/wk) at 0.03 mg/m^3 for up to 52 wk (the latter group actually received 0.1 mg/m^3, 6.5 h/d and 0.0025 mg/m^3 for the remaining 17.5 h/d for a time-weighted average exposure of 0.029 mg/m^3 over a 24-h period; the study author referred to this latter group as the 0.1 mg/m^3 exposure group). Ocular effects including corneal opacities, pannus, chronic keratitis, vascularization, pigmentation and granulation were the only overt signs of toxicity observed in the course of the study, and were only observed in dogs in the 0.1 mg/m^3 exposure group. Clinical chemistry analysis revealed only a slight increase in serum glutamic oxaloacetic transaminase (SGOT) activity in the high-dose dogs, which was of no biologic consequence. Three of 10 dogs exposed at 0.1 mg/m^3 exhibited chronic keratitis and conjunctivitis that was considered to be treatment related following prolonged exposure (7.5 or 12 mo) to sulfur mustard. In addition, there was no evidence of respiratory sensitization in the sulfur-mustard-exposed dogs. Because the study did not provide acute exposure-response data and involved long-term, repeated exposures not consistent with the exposure scenarios for AEGL application, the data are not directly applicable to the development of AEGL values. However, the results of this long-term exposure study may be useful as reference points to assess the validity of AEGLs.

3.2.2. Rats

McNamara et al. (1975) conducted long-term inhalation studies of sulfur mustard in Sprague-Dawley-Wistar rats. In the experiments, groups of rats (gender not specified) were exposed continuously at 0.001 mg sulfur mustard per cubic meter or discontinuously (6.5 h/d, 5 d/wk) at 0.1 mg/m^3

(see Section 3.2.1) for up to 52 wk. In the 79 rats exposed at 0.1 mg/m^3, there were no compound-related overt signs of toxicity. Necropsy revealed keratitis, possibly compound-related, in five of the rats. Necropsy revealed squamous cell carcinomas (skin) considered treatment related in four rats and squamous or basal cell carcinomas considered possibly treatment related in five rats (see Section 3.5).

Anderson et al. (1996) reported on the pathologic changes in adult male rats following 50-min intratracheal administration of sulfur mustard (0.35 mg/100 μL absolute ethanol). The dose of sulfur mustard was selected based on preliminary studies (data not provided) indicating that such an exposure would produce consistent but nonlethal damage at 24 h postexposure. Controls were treated similarly with absolute ethanol without the involvement of sulfur mustard. During exposure, the rats were anesthetized with Ketamine and they were euthanized at 0, 1, 4, 6, 12, 18, or 24 h postexposure. At 6 h postexposure, gross pathology assessments revealed multifocal petechial hemorrhages on the pleural surface of the lungs. Atelectasis and edema of the accessory lobe and necrosis and sloughing of tracheal and bronchial epithelia were observed at 6–12 h postexposure. Analysis revealed that most histologically defined lesions were confined to the trachea, bronchi, and larger bronchioles rather than the pulmonary region. There were no findings in the control group and little or no effects were observed in the sulfur-mustard-treated rats during the first 4 h after exposure. A latent phase of 4–6 h following sulfur mustard exposure was required for development of histologic lesions (epithelial necrosis and sloughing). Lymphoid necrosis, loss of lymphocytes, and damage to tracheal cartilage were observed at 12 h postexposure. At 24 h postexposure, peribronchiolar and perivascular edema were detected, but small bronchioles and alveoli appeared to be unaffected, although they contained some cellular debris and inflammatory cells. Ultrastructural examination revealed an increased number of alveolar macrophages in some foci of mild edema at 6 h. At 12 h postexposure, injury to Type 1 pneumocytes was observed, and edematous material, cellular debris, extravasated erythrocytes, and fibrin were seen in scattered alveoli. Evidence of hyperplasia and hypertrophy of Type II pneumocytes was observed at 18–24 h postexposure. An actual administered concentration of sulfur mustard was not provided, and there were no provisions in the experimental apparatus for actual measurement of the test material. The results of this study are consistent with the pattern of respiratory tract injury observed in humans following low-level exposure to sulfur mustard. Because

the study did not provide acute exposure-response data and involved long-term, repeated exposures not consistent with the exposure scenarios for AEGL application, the data are not directly applicable to the development of AEGL values. However, the results of this long-term exposure study may be useful as a reference point to assess the validity of AEGLs.

3.2.3. Mice

In the long-term inhalation study by McNamara et al. (1975), groups of A/J mice were exposed to sulfur mustard at 0.001 mg/m^3 continuously or discontinuously (6.5 h/d, 5 d/wk) at 0.1 mg/m^3 (see Section 3.2.1) for up to 52 wk. There were no overt signs of toxicity in the exposed mice during the treatment period. Deaths occured among the mice, but the investigators attributed those to adverse temperature extremes in the animal quarters, not to cumulative Ct for sulfur mustard. No clinical chemistry analyses were performed on the mice. There were no treatment-related tumors in mice exposed to sulfur mustard at 0.1 mg/m^3 (see Section 3.5). Because the study did not provide acute exposure-response data and involved long-term, repeated exposures not consistent with the exposure scenarios for AEGL application, the data are not directly applicable to the development of AEGL values. However, the results of this long-term exposure study may be useful as reference points to assess the validity of AEGLs.

Groups of four adult female Swiss mice (24–26 g) were exposed to sulfur mustard (>99% purity) at concentrations of 8.5, 16.9, 21.3, 26.8, 42.3, or 84.7 mg/m^3 for 60 min (Vijayaraghavan 1997). A group of mice exposed to filtered air for 60 min served as untreated controls, and mice exposed to acetone vapor served as vehicle controls. In this head-only exposure study, respiratory patterns of the mice were monitored for 7 d, and the animals were observed for up to 14 d postexposure. Sulfur mustard vapor was generated using a known quantity of sulfur mustard diluted with acetone and pumped into a compressed air nebulizer. Pressure in the nebulizer was adjusted for complete evaporation of the acetone diluent. A constant air flow of 20 L/min was maintained in the 50 cm×10 cm exposure chamber. The chamber air was sampled at a rate of 50 mL/min for 5 min and analyzed by gas chromatography (flame ionization detector). At 15–20 min into the exposure, the mice exposed to sulfur mustard exhibited signs of sensory irritation and their respiratory rate progressively decreased until 30 min into the exposure after which no further decrement was de

tected. The RD_{50} was calculated to be 27.4 mg/m^3. By postexposure day 1, there was a concentration-dependent decrease in respiratory rate over the 7-d monitoring period that was statistically significant ($p<0.05$) for the 21.3, 26.8, and 42.3 mg/m^3 groups relative to the unexposed controls. Decreases were as much as 40–60% of controls in the three exposure groups. Respiratory rate was also notably decreased (64.8% of that of controls) in the 16.9 mg/m^3 group, but the change was not statistically significant. Although exposure-response data were not provided, lethality was reported for mice in the "higher exposure" groups until 6 d postexposure.

Kumar and Vijayaraghavan (1998) provided additional information regarding nonlethal responses of mice to inhaled sulfur mustard. Groups of 30 female albino mice were exposed (head-only) for 1 h to sulfur mustard at concentrations of 21.2, 42.3, or 84.6 mg/m^3 (equivalent to 0.5, 1.0, and 2.0 LC_{50}) and sacrificed at 6, 24, or 48 h or 7 d after exposure. The exposure system was as previously described by Vijayaraghavan (1997). Even at the highest exposure, no mice died during exposure, although the mice did exhibit sensory irritation resulting in pauses between inspiration and expiration and decreased ventilatory frequency. Effects of sulfur mustard exposure on blood uric acid and urinary uric acid were also examined as an index of purine catabolism. Exposure to sulfur mustard at all concentrations tested resulted in significant increases in blood uric acid and urinary uric acid at all time points measured (except the 6-h time point for the low-dose group). The greatest concentration appeared to be at 24 h and generally decreased, although not to control levels, by 7 d. The increased blood uric acid was postulated as the result of catabolism of apurinated bases resulting from DNA adduct formation by sulfur mustard.

3.2.4. Rabbits

In an early study by Warthin and Weller (1919), rabbits (no information provided regarding gender, age, weight, or strain) were exposed to sulfur mustard at various concentrations and for various periods of time. The sulfur mustard concentrations were determined based on changes in weight of the sulfur mustard sample and the air flow and were simply expressed as ratios. The exposure regimen for eight rabbits and their respective responses are summarized in Table 2–5. The study authors concluded the following: (1) respiratory lesions are proportional to the concentration

TABLE 2-5 Effects on Rabbits of Acute Inhalation Exposure to Sulfur Mustard

Rabbit Number	Exposure[a]	Effects
32	58 mg/m^3 (1:110,000); 40 min	Signs of mild ocular and nasal irritation during exposure; increasing severity of conjunctival erythema and lacrimation up to sacrifice at 12 h; pulmonary congestion and edema
33	389 mg/m^3 (1:15,000); 20 min	Mild irritation during exposure; increased lacrimation and marked erythema of nostrils, mouth, ears, conjunctiva, and some dermal areas up to sacrifice at 36 h; evidence of edema and necrosis in nasal passages
30	389 mg/m^3 (1:15,000); 30 min	Signs of ocular irritation within 5 min after exposure; increased severity of ocular involvement progressing to extreme conjunctival edema and corneal ulceration; evidence of respiratory involvement by day 2; no increase in severity at time of sacrifice (4.25 d); marked congestion and edema in all areas of respiratory tract
31	214 mg/m^3 (1:30,000); 35 min	Minor nasal and ocular irritation immediately following exposure period that increased in severity up to sacrifice at 30 h; congestion in all areas of respiratory tract
46	130 mg/m^3 (1:50,000); 6 h	Signs of irritation during exposure; dead at 60 h postexposure (likely due to *Staphylococcus* infection)
45	130 mg/m^3 (1:50,000); 6 h	Similar effects and cause of death as noted for rabbit number 46
43	130 mg/m^3 (1:50,000); 12 h	Signs of ocular and nasal irritation, and lethargy during exposure; dead at 54 h postexposure; marked respiratory tract involvement and secondary infection in larynx and trachea
44	130 mg/m^3 (1:50,000); 12 h	Severe ocular effects and generalized dermal burns; congestion and necrosis in respiratory tract; congestion in other organs; secondary *Staphylococcus* infection involvement; sacrificed at 92 h postexposure

[a]Values in parentheses are the dilutions as reported by Warthin and Weller (1919).

and the length of exposure; (2) effects are mild following 10–15 min exposures at dilutions of 1:110,000 (58 mg/m^3) or following one to several exposures at higher concentrations; (3) nasal irritation is almost immediate and is followed by moderate ocular effects (photophobia, lacrimation) within 2–3 h and respiratory involvement at 2–3 h; (4) for prolonged or high-concentration exposures, pronounced respiratory effects occur somewhat later than ocular effects; (5) there are concentration- and time-dependent effects on severity of gross and histopathologic lesions such that long exposures or exposures to high concentrations will result in deeper tissue damage and damage to pulmonary regions, in addition to nasopharyngeal regions, and may increase susceptibility to secondary infection.

Rabbits exposed continuously to sulfur mustard at 0.001 mg/m^3 or discontinuously (6.5 h/d, 5 d/wk) at 0.1 mg/m^3 (see Section 3.2.1) for up to 52 wk exhibited no overt signs of toxicity (McNamara et al. 1975). Ocular sensitization tests were also performed on rabbits; the results were negative.

The effect of sulfur mustard vapor on rabbit eyes was examined by Laughlin (1944). In that study, rabbits were exposed to sulfur mustard (200–1,200 mg·min/m^3) for 30 or 60 min and observed for 24 h. Further details regarding experimental protocol are unavailable. Laughlin provided the following observations: redness and conjunctival edema but no corneal damage at 200 mg·min/m^3; some corneal opacity but no conjunctival discharge at 400 mg·min/m^3; excessive lacrimation with no purulent discharge at 600 mg·min/m^3; purulent discharge at 800 mg·min/m^3; and severe conjunctival edema at 1,200 mg·min/m^3. It was also reported that, for ocular effects, a Ct delivered over a 2-min period resulted in a more severe effect than the same Ct delivered over a 30-min or 60-min period and when the exposure duration was extended to 7 h, the severity of the effect was diminished (i.e., the 7-h Ct needed to be twice the 30- or 60-min Ct to obtain an equivalent effect). These observations imply that the concentration becomes less important over time and that there may be some form of a detoxification or recovery mechanism regarding ocular effects (Laughlin 1944; McNamara et al. 1975).

3.2.5. Guinea pigs

In the long-term inhalation study by McNamara et al. (1975), guinea pigs were used to assess the sensitization potential of sulfur mustard. For

this phase of the study, the guinea pigs were exposed to sulfur mustard at 0.001 mg/m^3 continuously or discontinuously (6.5 h/d, 5 d/wk) at 0.1 mg/m^3 (see Section 3.2.1) for up to 52 wk. Groups of six animals were removed after 1, 2, 4, 8, 32, and 52 wk of exposure. There was no evidence of sensitization in any of the group following challenge with a 7.9-μg dermal application of sulfur mustard in olive oil. The challenge had been previously shown to induce erythema, edema, and necrosis in sensitized animals. Dermal application of sulfur mustard at 31.6 μg or 63.2 μg (shown to induce a response in normal animals) to the same guinea pigs produced responses similar to those of controls, indicating that a tolerance had not been developed. Respiratory patterns were also examined during the sensitization tests and found to be unaffected by the treatment. No other treatment-related effects were reported for the guinea pigs.

The effects of sulfur mustard injected intratracheally (0.3 mg/kg; equivalent to approximately 0.6 mg sulfur mustard per cubic meter based on a body weight of 0.84 kg and ventilatory rate of 0.40 m^3/d) into male Hartley guinea pigs were studied by Calvet et al. (1994). In the study, guinea pigs (five per group) received a single intratracheal injection. Lung mechanics, airway responsiveness, microvascular permeability, and neutral endopeptidase activity in tracheal epithelium were assessed 5 h and 14 d after administration of the test article. At 5 h postinjection there was a 3-fold increase in respiratory system resistance ($p<0.05$) and a 2-fold increase in microvascular permeability ($p<0.05$). Histopathologic findings included shedding of tracheal epithelium columnar cells and peribronchial edema. At 14 d postinjection, the guinea pigs exhibited airway hyperactivity to inhaled substance P (an endogenous vasoactive peptide) and histamine.

3.3. Neurotoxicity

There are no data available regarding the neurotoxic effects of inhaled sulfur mustard in animals.

3.4. Developmental and Reproductive Toxicity

In the McNamara et al. (1975) study, groups of 10 female rats were exposed to sulfur mustard at 0.001 or 0.1 mg/m^3 during the first, second, or third week of gestation or for the entire gestation period. No increase in

fetal abnormalities was observed, and the fetal mortality rate was also within normal limits.

3.5. Genotoxicity

The potential genotoxicity of sulfur mustard was also examined by McNamara et al. (1975). Groups of 10 female rats were bred to males that had been exposed to sulfur mustard at 0.001 or 0.1 mg/m^3 for 1, 2, 4, 8, 12, 24, 36, or 52 wk. Based on number of live or dead fetuses and implantation sites, there was no evidence of dominant lethal mutagenesis.

3.6. Chronic Toxicity and Carcinogenicity

Animal carcinogenicity data have been summarized before in IARC (1975), Watson et al. (1989), IOM (1993), and USACHPPM (2000).

In a study reported by Heston and Levillain (1953), groups of 40 male and 40 female Strain A mice (2–3 mo old) were exposed for 15 min to sulfur mustard (0.01 mL) in an 8-liter desiccator while an equivalent number of control mice were exposed to air alone. At 4 mo after exposure, 30 test mice and 32 control mice were killed, and the lung tumor incidences were found to be 9/30 and 6/32. The remaining mice were killed at 11 mo postexposure, and the total tumor incidences (tumor type not specified) were found to be 33/67 and 21/77 for the treated and control groups, respectively. The incidences were significantly different at $p<0.01$. The number of tumors per mouse was 0.66 and 0.31 in the treated and control groups, respectively.

McNamara et al. (1975) provided evidence of the tumorigenic potential of long-term exposure to sulfur mustard in Sprague-Dawley-Wistar rats. Seventy male and 70 female rats were continuously exposed to sulfur mustard at 0.001 mg/m^3 for 24 h/d, 5 d/wk, or at 0.1 mg/m^3 for 6.5 h/d followed by 0.0025 mg/m^3 for 17.5 h/d, 5 d/wk, for up to 12 mo. Both gross and microscopic examinations were conducted on major tissues and organs. Fifty subjects of each gender were maintained as controls. Results of this toxicity study are shown in Table 2–6. Lesions considered agent-related included squamous cell carcinomas and basal cell carcinomas of the skin.

EPA (1991) emphasized that the studies of McNamara et al. (1975) contain deficiencies that make a quantitative analysis difficult. The studies

were conducted in 1970. They do not conform to current standards of experimental protocol and likely contain bias in the assignment of animals to test categories. In addition, many of the exposures were very brief and included only a few animals, many of which were sacrificed (and some were replaced) before their capacity to develop late-appearing tumors could be fully tested. Despite these shortcomings, EPA (1991) noted that the McNamara et al. (1975) data are the best available for directly estimating the carcinogenic potency of sulfur mustard.

TABLE 2–6 Rat Skin Tumor Data from the McNamara et al. (1975) Toxicity Study[a]

	Exposure Groups		
Gender	Control	Low Exposure[b]	High Exposure[c]
Males	0/11	0/10	4/11
Females	0/8	0/19	5/18
Both genders	0/19	0/29	9/29

[a]Includes only data for rats living longer than the time until first tumor appearance (12 mo exposure plus 70 d postexposure).
[b]0.001 mg/m^3 for 24 h/d, 5 d/wk.
[c]0.1 mg/m^3 for 6.5 h/d followed by 0.0025 mg/m^3 for 17.5 h/d, 5 d/wk.
Source: EPA 1991.

In addition, a study specifically addressing carcinogenic potential was also conducted by McNamara et al. (1975) in which groups of rats were exposed for varying time periods up to 21 mo to the same sulfur mustard concentrations as used in the toxicity study. The animals were then observed for varying periods of time before being sacrificed. As is the case for the toxicity study, both gross and microscopic examinations of major tissues and organs were conducted in the carcinogenicity study. The results of the study are shown in Table 2–7. Agent-related lesions included squamous cell and basal cell carcinomas of the skin, trichoepitheliomas of the skin, and keratoacanthomas of the skin.

McNamara et al. (1975) also conducted carcinogenicity studies in ICR Swiss albino as well as strain A/J mice, dogs, rabbits, and guinea pigs exposed to the same sulfur mustard concentration protocols as in the previously described toxicity study for varying exposure durations up to 1 y. Necropsy protocols were the same as for the rat toxicity and carcinogenic

ity studies. No exposure-related tumors were observed in any of the species.

TABLE 2–7 Rat Skin Tumor Data from McNamara et al. (1975) Cancer Study, By Increasing Lifetime Daily Exposure

Exposure Duration (wk)	Exposure Concentration[a]	Lifetime[b] Average Daily Exposure (μg/m^3)	Incidence of Skin Carcinomas
Control	0	0.0	0/27
1	Low	0.0096	0/5
2	Low	0.0192	0/5
4	Low	0.0385	0/5
8	Low	0.0769	0/4
12	Low	0.115	0/5
26	Low	0.250	0/4
1	High	0.279	0/5
39	Low	0.375	0/3
52	Low	0.500	0/17
2	High	0.558	0/5
4	High	1.12	0/6
8	High	2.23	0/4
12	High	3.35	4/5
26	High	7.25	4/5
39	High	10.9	4/4
52	High	14.5	10/23

[a]Low exposure was 0.001 mg/m^3 24 h/d, 5 d/wk; high exposure was 0.1 mg/m^3 for 6.5 h/d followed by 0.0025 mg/m^3 for the remaining 17.5 h/d, 5 d/wk.
[b]A 2-y lifetime was assumed
Source: EPA 1991.

A recent comparative analysis evaluated the tumorigenicity of sulfur mustard relative to alkylating compounds used in chemotherapy or treatment of other diseases (Nicholson and Watson 1993). By considering all possible combinations of experiments and several reference compounds, sulfur mustard tumorigenicity was determined to be comparable to nitrogen

mustard (HN2 and HN2-HCl) tumorigenicity in laboratory rodents. Additional relative potency comparisons were made for the therapeutic nitrogen mustards melphalan and chlorambucil and the alkylating carcinogenic compound bis (chloromethyl) ether. Comparisons of laboratory rodent data indicated that sulfur mustard and nitrogen mustard had tumorigenic potencies comparable to melphalan and bis(chloromethyl) ether; the tumorigenic potencies of sulfur and nitrogen mustard were possibly greater than that of chlorambucil (Nicholson and Watson 1993).

3.7. Summary

The available acute lethality data in animals are summarized in Table 2–8. Lethality data from earlier reports were not verifiable but are not totally inconsistent with those from later studies. For example, the 1-h LC_{50} values of 14.0 mg/m^3 and 14.3 mg/m^3 for rats and mice derived, respectively, from the 840 mg·min/m^3 and 860 mg·min/m^3 60-min LCt_{50} values reported by Fuhr and Krakow (1945) are similar to the lower confidence limit of the mouse 1-h LC_{50} (13.5 mg/m^3) reported by Vijayaraghavan (1997) (i.e., 13.5 mg/m^3). The values are also similar to a 1-h LC_{50} of 13.3 mg/m^3 for guinea pigs that can be extrapolated (assuming $C^l \times t = k$) from the 5-min LCt_{50} of 800 mg·min/m^3 reported by Langenberg et al. (1998). Anecdotal LCt_{50} values for the dog, cat, goat, and monkey were also reported by Rosenblatt et al. (1975). Those data are shown in Table 2–5, but details were unavailable for verification of the values. An overview of the data suggests that interspecies variability in the lethal response to sulfur mustard vapor is less than an order of magnitude.

Overall, the available animal data regarding nonlethal effects suggest that test species exhibit signs of toxicity that are qualitatively similar to those of humans when acutely exposed to sulfur mustard vapor. Ocular and respiratory tract irritation and the fact that those are primary targets are plainly evident in studies using dogs, rats, mice, rabbits, and guinea pigs. Long-term exposure of dogs, mice, and guinea pigs to concentrations at 0.03 mg/m^3 produced only minor signs of ocular and respiratory tract irritation, although similar exposures in rats were tumorigenic. One-hour exposure of mice to concentrations up to 16.9 mg/m^3 resulted in notable but not serious effects on respiratory parameters and acute exposures of rabbits (20 min to 12 h) to concentrations ranging from 58 mg/m^3 to 389 mg/m^3 ($Ct \geq 2{,}300$ mg·min/m^3) resulted in severe respiratory tract damage. There are no

data available regarding the neurotoxic effects of inhaled sulfur mustard toxicity in animals. Limited data in rats revealed no increase in fetal abnormalities or fetal mortality following exposure to sulfur mustard. The results of a single study in rats indicated no evidence of dominant lethal mutagenesis based on the numbers of live or dead fetuses and implantation sites.

TABLE 2–8 Acute Lethality of Sulfur Mustard in Laboratory Species

Species	Lethality Value	Concentration (mg/m^3) and Exposure Duration (min)	Reference
Rat	2-min LCt_{50}: 1,512 mg-min/m^3 30-min LCt_{50}: 990 mg-min/m^3 60-min LCt_{50}: 840 mg-min/m^3	756 mg/m^3 (2 min) 33 mg/m^3 (30 min) 14 mg/m^3 (60 min)	Fuhr and Krakow 1945 (not verified)
Mouse	2-min LCt_{50}: 4,140 mg-min/m^3 30-min LCt_{50}: 1,320 mg-min/m^3 60-min LCt_{50}: 860 mg-min/m^3	2,070 mg/m^3 (2 min) 44 mg/m^3 (30 min) 14.3 mg/m^3 (60 min)	Fuhr and Krakow 1945 (not verified)
Mouse	60-min LC_{50}:	42.5 mg/m^3 42.5 mg/m^3 (60 min)	Vijayaraghavan 1997
Monkey	10-min LCt_{50}: 800 mg-min/m^3	80 mg/m^3 (10 min)	Rosenblatt et al. 1975
Dog	10-min LCt_{50}: 600 mg-min/m^3	60 mg/m^3 (10 min)	Rosenblatt et al. 1975
Cat	10-min LCt_{50}: 700 mg-min/m^3	70 mg/m^3 (10 min)	Rosenblatt et al. 1975
Goat	10-min LCt_{50}: 1,900 mg-min/m^3	190 mg/m^3 (10 min)	Rosenblatt et al. 1975
Guinea pig	5-min LCt_{50}: 800 mg-min/m^3 10-min LCt_{50}: 1,700 mg-min/m^3	160 mg/m^3 (5 min) 170 mg/m^3 (10 min)	Langenberg et al. 1998; Rosenblatt et al. 1975

There are data indicating the tumorigenic potential of sulfur mustard in laboratory species following inhalation exposure. A tentative quantitative assessment of cancer risk for a single acute exposure is presented in Appendix C. That assessment, following the NRC methodology for EEGLs, SPEGLs, and CEGLs (NRC 1986), is based on a geometric mean of slope factors developed using various data sets and indicates an excess cancer risk of 1 in 10,000. The resulting 10^{-4} excess cancer risk values are similar to the AEGL-3 values, and 10^{-5} and 10^{-6} excess cancer risk values would be considerably lower than the AEGL-3s. The use of excess cancer risk estimates in setting AEGL values is precluded by the uncertainties involved in assessing excess cancer risk following a single acute exposure of 8-h or less duration, by the relatively small population exposed in an emergency release situation, and by the potential risks associated with evacuations.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

A thorough understanding of the metabolism and disposition of sulfur mustard is not likely to be pivotal in the quantitative assessment of human health risk from acute exposures. One of the most important aspects of the disposition of sulfur mustard is that its lipophilic nature allows for toxicologically significant quantities to penetrate the skin (Papirmeister et al. 1991). In addition, its extreme cytotoxicity is not dependent on metabolism and disposition, and its toxic potential to primary targets is not significantly ameliorated via detoxification processes. The stratum corneum of the skin offers the greatest barrier to penetration by sulfur mustard, and it is the absence of this layer that make the eyes and respiratory tract so susceptible to toxic insult from the compound.

Papirmeister et al. (1991) have reviewed available studies regarding the absorption and distribution of sulfur mustard. Although only a relatively small amount of sulfur mustard is absorbed following percutaneous application, experiments with radio-labeled material have shown distribution to most tissues within short periods of time (e.g., 15 min). Henriques et al. (1943) estimated that about 12% of a dose absorbed into the skin actually reacts with tissue components and that it is this portion of the dose that is responsible for the vesicant effects.

The toxicokinetics of sulfur mustard and its DNA adduct, N7-hydroxyethylthioethyl guanine (SM-7-gua), were studied by Langenberg et al. (1998) in hairless guinea pigs exposed via nose-only inhalation, percutaneous exposure to vapors, or intravenous injection of sulfur mustard. The time course for sulfur mustard in the blood of guinea pigs following a single intravenous injection of 1 or 0.3 LD_{50} (96-h intravenous LD_{50} =8.2 mg/kg) showed a rapid disappearance (>1,000-fold reduction) within 10 min and maintained this level or slightly less to 360 min. Overall, the toxicokinetics of intravenously administered sulfur mustard was biphasic and exhibited a very rapid distribution phase and a slow elimination phase. Significant partitioning of sulfur mustard into the lungs, liver, spleen, and bone marrow was also observed. At time points from 0.05 h to 48 h after intravenous administration, the concentration of SM-7-gua adducts (expressed per 10^7 nucleotides) was significantly greatest in the lung (10–400 adducts) but also detected (2–30 adducts) in all tissues examined (liver, spleen, bone marrow, small intestine, blood). Results of inhalation toxicokinetic studies using hairless guinea pigs exposed nose-only to 1 LCt_{50} for 5 min revealed sulfur mustard concentrations in the blood below detection limits (5 pg/ml). SM-7-gua adducts could not be detected in the spleen, bone marrow, or small intestine but very low levels (0.7 adducts per 10^7 nucleotides) were detected in the lung at 10 min and 48 h after exposure. Adducts were detected in the nasal, nasopharynx, larynx, trachea, and carina of the respiratory tract (50–80 adducts per 10^7 nucleotides) at 4 h after exposure. On the basis of these blood concentration and adduct distribution data the authors concluded that during acute inhalation exposure in guinea pigs most of the sulfur mustard reacts with upper airway tissues. For species with less complex nasal systems (such as humans), more sulfur mustard could conceivably reach the lungs.

Several studies have been conducted using intravenously administered ^{35}S-labeled sulfur mustard to assess metabolism and disposition. For intravenous studies in rabbits, Boursnell et al. (1946) reported that sulfur mustard was widely distributed and excreted primarily in the urine. The highest concentration of radio-label was detected in the lungs, liver, and kidneys. Similar excretory processes were observed for rats and mice. Results of these studies also identified thiodiglycol and conjugates, glutathione-bis-β-chloroethylsulphone conjugates, and bis-β-chloroethylsulphone and conjugates as urinary metabolites.

Studies using intravenously administered ^{35}S-labeled sulfur mustard (0.1 mg/ kg in ethanol) were also conducted in human terminal-cancer pa

tients (Davison et al. 1961). Within 24 h, 23% of the dose was excreted in the urine. Within 48 h, 27% was excreted in the urine. Based on chromatographic analysis, the metabolites were similar to those identified for rats and mice.

4.2. Mechanism of Toxicity

The principal mechanism of toxicity for sulfur mustard may be attributed to its capacity as an alkylating agent and consequent ability to react with DNA, RNA, and other macromolecules (reviewed by Watson and Griffin [1992]). Endothelial cells are a major target for sulfur mustard (Dabrowska et al. 1996). Because of the fundamental nature of these targets, the actual mechanism of toxicity may be complex. Cross-linking with DNA (Lohs 1975; Gross et al. 1985; Lin et al. 1996) and inhibition of enzymes such as hexokinase (Dixon and Needham 1946) have been reported, and sulfur mustard has been shown to be especially toxic to proliferating cells (Vogt et al. 1984; Gross et al. 1985). In addition, mechanisms such as the cell membrane modifications in the absence of DNA damage have been described (Levy 1934).

A hypothesis for the skin lesion and blistering effects of sulfur mustard has been provided by the U.S. Army Medical Research Institute of Chemical Defense (Papirmeister et al. 1985; Gross et al. 1985). This hypothesis contends that a depletion of NAD+ arising from efforts to repair extensive DNA damage results in inhibition of glycolysis. The inhibition of glycolysis stimulates the hexose monophosphate shunt, which causes a release of proteases that are instrumental in the skin damage associated with sulfur mustard exposure. More recently, Petrali and Oglesby-MeGee (1997) reported results from investigations using several animal models, cultured isolated human cells, and in vitro organotypic skin models. Histopathologic and ultrastructural analysis indicated that basal cells of the stratum basale layer is an early target of sulfur mustard and that resulting injury that is evident by 4–6 h after exposure represents a progressive and irreversible cell injury and death. In addition, there appears to be a disabling of anchoring hemidesmosome filaments resulting in microvesicle formation and interaction with various membrane proteins such that there is a loss of immunospecificity.

Using a chromogenic peptide substrate assay, Cowan et al. (1993) found that sulfur mustard enhanced proteolytic activity. A time-dependent

and temperature-dependent proteolysis was observed for in vitro experiments using human peripheral blood lymphocytes. A similar response was also seen for in vivo exposures using the hairless guinea pig.

In vitro experiments conducted by Smith et al. (1990) and Smith and Smith (1997) using primary human epidermal keratinocytes provided results showing a concentration-dependent interference with cell cycling. At concentrations equivalent to those that would produce vesication, the cell cycle was blocked at the G1-S interface, although at subvesicant concentrations, the cell cycle was blocked in the G2 phase.

Using bovine pulmonary artery endothelial cells, Dabrowska et al. (1996) showed that sulfur mustard ($\leq$250 μM) induced apoptosis within 5 h. At concentrations $\geq$500 μM both apoptotic and necrotic cell death occurred after 5–6 h. Necrosis was accompanied by a significant depletion of intracellular ATP.

Most sulfur-mustard-induced fatalities have been due to respiratory tract involvement. The mechanism of sulfur-mustard-induced pulmonary damage was studied by Anderson et al. (1997) using lavage fluid from rats in which sulfur mustard (0.35 mg) was intratracheally intubated for 50 min. At 1, 4, or 24 h after the treatment, the rats were euthanized and the lungs lavaged with physiologic saline. Lactate dehydrogenase and γ-glutamyltransferase were increased ($p \leq 0.05$) at all time points, and total protein was increased ($p < 0.001$) at 4 and 24 h. The investigators contended that these indices were useful indicators of early pulmonary injury following low-dose exposure to sulfur mustard.

4.3. Structure-Activity Relationships

There are no structure-activity data that would be instrumental in the development of AEGL values for sulfur mustard.

4.4. Other Relevant Information

There are several important aspects of sulfur mustard toxicology that impact the toxic response and are relevant to assessing human health risk. They include the latency period between initial exposure and development of effects, the effect of temperature and humidity, the variable sensitivity among tissues and sites affected, and the sensitization potential for vesicating effects. First, it is well documented (summarized by Papirmeister et al.

[1991]) that a latency period exists between the initial exposure to sulfur mustard and the development of toxic effects. That pertains not only to onset of effects but also to development of full severity of effects. The ocular response appears to have the shortest latent period, sometimes as short as minutes, whereas dermal and respiratory effects following acute exposure may take days for full development. It is also known that higher ambient temperature and greater humidity enhance the dermal response to sulfur mustard (Nagy et al. 1946; Renshaw 1947; Papirmeister et al. 1991). Although the mechanism is unknown, increased temperature and humidity decrease the dose required for a given response and increase the severity of the response. In this respect, moisture (in addition to skin characteristics) is relevant to the greater sensitivity of certain anatomical areas (e.g., axial, interdigital, and popliteal areas, scrotum, and perineum). The eyes and respiratory tract are generally considered the most sensitive organs/tissues (eyes somewhat more so) for acute exposures to sulfur mustard. Both involve latency periods and a wide range of severity of effects depending primarily on the exposure concentration, but injury to the respiratory tract is considered more relevant regarding lethal responses. Sensitization to sulfur-mustard-induced dermal effects appears to be associated with repeated exposures and, according to McNamara et al. (1975), occurs after detectable insult (i.e., overt clinical signs). There tends to be a greater sensitivity to high exposures but no greater severity in response to lower exposures or greater likelihood of a response to lower exposures (Sulzberger et al. 1945).

4.4.1. Species Variability

All of the species tested exhibit qualitatively similar responses to sulfur mustard vapor and affirm that the eyes and respiratory tract are the most sensitive targets. Available lethality data (LC_{50} and LCt_{50}) are remarkably similar across species (see Section 3.1.4).

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

Walker et al. (1928) reported that four of seven men exposed to sulfur mustard at 0.001 mg/L (1 mg/m^3) for 5–45 min exhibited conjunctivitis, and

two exhibited skin burns. It was also reported that, of 17 men exposed at 0.0005 mg/L (0.5 mg/m^3) for 10–45 min (5–22.5 mg·min/m^3), six exhibited conjunctivitis, and one had a skin burn. Three of 13 men exposed for 10–30 min at 0.0001 mg/L (0.1 mg/m^3; Ct of 1–3 mg·min/m^3) showed slight but distinct conjunctivitis. Although not of a severity consistent with an AEGL-2 level, those effects are of greater severity than would be acceptable for AEGL-1 development. Guild et al. (1941) also conducted experiments using humans and reported that (1) exposure to Ct values <70 mg·min/m^3 would result in mild conjunctival responses that would not be indicative of a casualty (temporary loss of vision); (2) Ct values of 70–100 mg·min/m^3 would produce some casualties and; (3) Ct values >100 mg·min/m^3 would be expected to produce disabling ocular effects of several days' duration. Because the subjects wore respiratory protection, effects on the respiratory tract could not be determined.

In experiments with human volunteers exposed to varying concentration-time regimens, Anderson (1942) found that an exposure concentration-time product of 12 mg·min/m^3 was without effects and 30 mg·min/m^3 represented the upper range for mild effects (conjunctival injection and minor discomfort with no functional decrement). Ct products slightly higher than that (e.g., 34–38.1 mg·min/m^3) were, however, also without appreciable effects, thereby indicating that the response to 30 mg·min/m^3 is consistent with AEGL-1 effects.

Odor thresholds of 1 mg·min/m^3 (Bloom 1944), 0.15 mg/m^3 (Ruth 1986) and 0.6 mg/m^3 (Dudley and Wells 1938; Bowden 1943; Fuhr and Krakow 1945) have been reported.

Analysis of the exposure-effect values from the human studies indicated that the 12-mg·min/m^3 value represented a defensible estimate of the threshold for effects consistent with the AEGL-1 definition. The 12-mg·min/m^3 exposure was without a symptomatic effect and, therefore, provides the basis for protective AEGL-1 values consistent with the AEGL-1 definition.

5.2. SUMMARY OF ANIMAL DATA RELEVANT TO AEGL-1

The effects described in the animal studies tend to be a of greater severity than those associated with AEGL-1 (i.e., signs of severe ocular irritation, body weight loss, respiratory depression, evidence of respiratory tract histopathology, etc.). There were no definitive exposure-response data in

animals that were considered appropriate for the development of AEGL-1 values.

5.3. Derivation of AEGL-1

The most tenable AEGL-1 values were developed using data reported by Anderson (1942) in which three to four human volunteers were exposed to agent HD at varying concentration-time regimens. In an analysis of those data, Anderson found that an exposure concentration-time product of 30 mg·min/m^3 represented the upper range for mild effects (conjunctival injection and minor discomfort with no functional decrement) and that 12 mg·min/m^3 represented a threshold for such effects. The 12 mg·min/m^3 represents a defensible estimate of the threshold for AEGL-1 effects. The 12-mg·min/m^3 exposure resulted in only minor conjunctival injection and no sensation of irritation. Ocular effects appear to be the most sensitive indicator of sulfur mustard exposure and toxicity, thereby justifying ocular irritation as an appropriate end point for development of AEGL values. All of the data considered were from human subjects, and, therefore, the uncertainty factor (UF) application to the 12-mg·min/m^3 value was limited to 3 for protection of sensitive individuals. The adjustment is considered appropriate for acute exposures to chemicals whose mechanism of action primarily involves surface contact irritation of ocular and/or respiratory tract tissue rather than systemic activity that involves absorption and distribution of the parent chemical or a biotransformation product to a target tissue. In addiction, Anderson (1942) noted that there was little variability in the ocular responses among the individuals participating in the study. That the AEGL-1 values are based on a sensitive end point is also reflected in that they are below reported odor thresholds (0.6 mg/m^3 and 1 mg·min/m^3).

Because exposure-response data were unavailable for all of the AEGL-specific exposure durations, temporal extrapolation was used in the development of AEGL-1 values for the AEGL-specific time periods. The concentration-exposure time relationship for many irritant and systemically acting vapors and gases can be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al. 1986). Analysis of available data regarding AEGL-1 type effects reported by Reed (1918), Reed et al. (1918), Guild et al. (1941), and Anderson (1942) indicate that for the exposure periods up to several hours, the concentration-exposure time relationship is a near-linear function (i.e., Haber's law where n=1 for $C^n \times t = k$) as

shown by *n* values of 1.11 and 0.96 for various data sets consistent with AEGL-1 effects (Appendix B). Therefore, an empirically derived, chemical-specific estimate of *n*=1 was used, rather than a default value, based on the ten Berge (1986) analysis. The derivation of the exponent *(n)* utilized human response data where 75–100% of the responders showed a mild response that would be consistent with the definition of AEGL-1 effects. In addition, the data provided by Anderson (1942) were indicative of a linear concentration-time relationship. The AEGL-1 values developed using the 12-mg·min/m^3 exposure value reported by Anderson (1942) are shown in Table 2–9. The AEGL-1 values are below the odor threshold for sulfur mustard (0.6 mg/m^3 and 1 mg·min/m^3).

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

Quantitative data regarding the human experience and AEGL-2 level effects are limited to responses ranging from signs of mild ocular irritation to ocular irritation that impairs normal visual function. Reed (1918) reported that 20–45 min exposure of himself and a volunteer at 1.2 mg/m^3 resulted in severe ocular irritation and dermal lesions. In a report of a subsequent experiment, Reed et al. (1918) noted that exposure of human volunteers at 0.1–4.3 mg/m^3 for 5–45 min produced ocular irritation and skin burns (0.5 mg/m^3 for 30 min) and very severe conjunctivitis, photophobia, skin burns, and nasopharyngeal exfoliation (1.0 mg/m^3 for 45 min). The analytical techniques used in these experiments were suspect; actual exposures were likely 30–40% higher. The report by Guild et al. (1941) of human exposure experiments did not provide findings of effects consistent with the AEGL-2 definition. Anderson (1942) reported on a series of human exposures resulting in varying degrees of ocular responses ranging from nonsymptomatic ocular injection to ocular irritation that required medical treatments and was considered severe enough to impair normal function.

6.2. Summary of Animal Data Relevant to AEGL-2

With the exception of a study reported by Warthin and Weller (1919) regarding the effects in rabbits following acute exposure, there is little

exposure-response data for animals consistent with AEGL-2-severity effects. Weller and Warthin reported severe ocular effects and dermal burns in rabbits exposed for 12 h to sulfur mustard at 130 mg/m^3. That study, however, was compromised by the use of single animals and lacks detail. Kumar and Vijayaraghavan (1998) reported alterations in purine catabolism in mice exposed for 1 h to sulfur mustard at 21.2–84.6 mg/m^3, but those exposures also represented 0.5, 1.0, and 2.0 LC_{50} responses. Statistically significant reductions in body weights were also observed for the mice at 14 d following a 1-h exposure to concentrations at 16.9–42.3 mg/m^3; however, at least some of the exposures were also associated with lethality. Dogs, rats, mice, and guinea pigs exposed continuously to sulfur mustard at 0.001 mg/m^3 or discontinuously (6.5 h/d, 5 d/wk) at 0.1 mg/m^3 for up to 52 wk did not exhibit effects consistent with the AEGL-2 definition (McNamara et al. 1975).

TABLE 2–9 AEGL-1 Values for Sulfur Mustard (ppm [mg/m3])a

10-min	30-min	1-h	4-h	8-h
0.06 (0.40)	0.02 (0.13)	0.01 (0.067)	0.003 (0.017)	0.001 (0.008

[a]The AEGL-1 values are at or below the odor threshold for sulfur mustard.

6.3. Derivation of AEGL-2

The AEGL-2 values for sulfur mustard were developed using data from Anderson (1942). The study utilized three or four human volunteers exposed to varying concentrations of sulfur mustard (1.7–15.6 mg/m^3) for time periods varying from 2 to 33 min. Anderson considered a Ct value of 60 mg·min/m^3 as the lowest concentration-time product for which ocular effects could be characterized as military casualties and that personnel exposed might be ineffective for up to (but no more than) 7 d. Effects included irritation, soreness, and widespread conjunctivitis, frequently accompanied by chemosis and photophobia. The 60-mg·min/m^3 exposure was used as the basis for developing the AEGL-2 values because it is representative of an acute exposure causing an effect severe enough to impair normal visual function and, although not irreversible, would certainly result in potential for additional injury. The ocular irritation and damage were also considered appropriate as a threshold estimate for AEGL-2 effects, because

the eyes are generally considered the most sensitive indicator of sulfur mustard exposure, and irritation would likely occur in the absence of vesication effects and severe pulmonary effects. The fact that the AEGL-2 is based on human data precludes the use of an interspecies UF. A factor of 3 was applied for intraspecies variability (protection of sensitive populations). The factor was limited to 3 under the assumption that the primary mechanism of action of sulfur mustard involves a direct effect on the ocular surface and that the response will not vary greatly among individuals (as noted by Anderson [1942]). A modifying factor of 3 was applied to accommodate potential onset of long-term ocular or respiratory effects. It was justified by the absence of long-term follow-up in the subjects of the Anderson (1942) study to confirm or deny development of permanent ocular or respiratory tract damage. Because the factors of 3 each represent a logarithmic mean (3.16) of 10, their product is 3.16×3.16=10. Further reduction by the application of additional modifying factors was not warranted because of the use of a sensitive indicator representing an AEGL-2 effect of marginal severity. As is the case for AEGL-1 values, time scaling was conducted using an n of 1 for all time points (Appendix B). The resulting AEGL-2 values are shown in Table 2–10, and their derivation is presented in Appendix A. Similar to the AEGL-1 values, all of the AEGL-2 values are at or below the reported odor thresholds (0.6 mg/m^3 and 1 mg·min/m^3).

TABLE 2–10 AEGL-2 Values for Sulfur Mustard (ppm [mg/m3])a

10-min	30-min	1-h	4-h	8-h
0.09 (0.60)	0.03 (0.20)	0.02 (0.10)	0.004 (0.025)	0.002 (0.013)

[a]The AEGL-2 values are at or below the odor threshold for sulfur mustard.

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

Human lethality data are limited to an inhalation LCt_{50} estimate of 1,500 mg·min/m^3 and percutaneous LCt_{50} estimate of 10,000 mg·min/m^3 estimated from animal data (DA 1974). The NRC (1997) concluded that an estimated LCt_{50} for humans of 900 mg·min/m^3 developed by the U.S.

Army based on an average of animal LCt_{50} data was scientifically valid but was developed in reference to healthy male military personnel and does *not* apply to civilians.

7.2. Summary of Animal Data Relevant to AEGL-3

Various lethality values have been reported for laboratory species acutely exposed to sulfur mustard. Vijayaraghavan (1997) reported a 1-h LC_{50} of 42.5 mg/m^3 for mice (head-only exposure). In a follow-up study reported by Kumar and Vijayaraghavan (1998), 1-h exposure of mice at 21.2 mg/m^3 did not result in lethality. Lethality estimates were based on deaths occurring up to 14 d after exposure. Langenberg et al. (1998) reported a 5-min LCt_{50} of 800 mg·min/m^3 for rabbits (deaths determined up to 96 h after exposure). These studies utilized up-to-date exposure and analytical systems and provided lethality estimates based on adequate numbers of animals evaluated at postexposure time frames appropriate for the known latency in sulfur-mustard-induced lethality.

7.3. Derivation of AEGL-3

As noted in Section 3.1.4, the lethality data from earlier reports were not verifiable but are not inconsistent with those from later studies. The 1-h LC_{50} values for rats and mice derived from the 840 and 860 mg·min/m^3 60-min LCt_{50} values reported by Fuhr and Krakow (1945) are similar to the lower confidence limit of the mouse 1-h LC_{50} reported by Vijayaraghavan (1997) (i.e., 14.0,14.3, and 13.5 mg/m^3, respectively; the corresponding Ct values are 840, 858, and 810 mg·min/m^3). The values are also similar to a 1-h LC_{50} of 13.3 mg/m^3 for guinea pigs extrapolated (assuming $C^1 \times t = k$) from the 5-min LCt_{50} of 800 mg·min/m^3 reported by Langenberg et al. (1998). However, the values from the earlier studies are not verifiable. In the inhalation toxicity study by Vijayaraghavan (1997), mice were exposed (head only) for 60 min to sulfur mustard at concentrations of 0.0, 8.5,16.9, 21.3, 26.8, 42.3 or 84.7 mg/m^3. The study investigator derived a 60-min LC_{50} of 42.5 mg/m^3 based on lethality at 14 d postexposure (95% confidence interval: 13.5–133.4 mg/m^3). In a follow-up study (Kumar and Vijayaraghavan 1998), there was no mortality in mice exposed at 0.5 LC_{50} (21.2 mg/m^3). Therefore, the 1-h exposure at 21.2 mg/m^3 was selected as an estimate of the lethality threshold in mice.

When compared with the human exposure-effect data, the 21.2-mg/m^3 concentration (Ct of 1,272 mg·min/m^3 for a 60-min exposure) is not an exposure that has been associated with lethality in humans (see Section 2.1). An intraspecies UF of 3 was applied for protection of sensitive individuals. This adjustment was considered appropriate for acute exposures to chemicals whose mechanism of action primarily involves surface contact irritation of ocular and/or respiratory tract tissue rather than systemic activity that involves absorption and distribution of the parent chemical or a biotransformation product to a target tissue. An interspecies UF was limited to 3 because available data do not suggest that humans are notably more sensitive than animals regarding lethality from inhalation exposure to sulfur mustard. The mechanism of pulmonary injury leading to lethality appears to be a function of the direct contact of an alkylating agent with epithelial tissue. This mechanism is likely to be more similar than different across mammalian species. Furthermore, the AEGL-3 values resulting from the aforementioned complement of UFs (total UF adjustment was 10; see Section 6.3) are equivalent to exposures known to cause only mild ocular effects in humans. The modifying factor of 3 utilized in the development of AEGL-2 values to account for uncertainties regarding the latency and persistence of the irritant effects of low-level exposure to sulfur mustard was not applied for AEGL-3 because lethality of the mice was assessed at 14 d postexposure in the key studies by Vijayaraghavan (1997) and Kumar and Vijayaraghavan (1998).

For derivation of the AEGL-3 values, there was uncertainty regarding the validity of applying linear extrapolation based on ocular effects to concentration-time extrapolations for lethality. As reported by ten Berge et al. (1986), the concentration-time relationship for many irritant and systemically acting vapors and gases can be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5. Therefore, in the absence of chemical-specific lethality data, time scaling was performed using exponential extrapolation (n=3) for shorter time periods and linear extrapolation (n=1) for longer time periods, thereby providing a somewhat more conservative (i.e., protective) estimate of the AEGL-3 values than would be obtained using an n value based on ocular irritation. The AEGL-3 values were derived by scaling from the 1 -h LC_{50} of 21.2 mg/m^3 reported by Kumar and Vijayarhagavan (1998) using $C^n \times t = k$ where n=1 or 3 (Appendix A). The concentration-time constant, k, was 1,272 mg·min/m^3 where n=1 and 571,687.68 mg·min/m^3 where n=3. The AEGL-3 values are shown in Table 2–11, and their derivation is presented in Appendix A The 4-h and 8-h AEGL-3 values are at or below reported odor thresholds.

TABLE 2–11 AEGL-3 Values for Sulfur Mustard (ppm [mg/m3])

10 min	30 min	1 h	4 h	8 h
0.59 (3.9)	0.41 (2.7)	0.32 (2.1)	0.08 (0.53)	0.04 (0.27)

Note: The 4-h and 8-h AEGL-3 values are below the odor threshold for sulfur mustard.

When comparing the Ct values generated by the draft AEGL-3 numbers with the human exposure data, any further reduction appears indefensible. The Ct values resulting from the AEGL-3 numbers (i.e., 39–130 mg·min/m^3) are similar to cumulative exposures shown to cause only ocular irritation in humans (Guild et al. 1941; Anderson 1942) and are similar to the ECt_{50} of 100 mg·min/m^3 for severe ocular effects (for soldiers) determined by Reutter and Wade (1994) and the NRC (1997). Furthermore, the AEGL-3 values are nearly similar to those developed using the human lethality estimate of 900 mg·min/m^3 (Reutter and Wade 1994) that was derived from multiple-species animal data, and reviewed by the NRC (1997). Assuming a 3-fold reduction for estimation of a lethality threshold ([900 mg·min/m^3] /3=300 mg·min/m^3) and another 3-fold reduction for consideration of sensitive populations ([300 mg·min/m^3]/3=100 mg·min/m^3), the resulting AEGL-3 values from the Reutter and Wade (1994) and NRC (1997) reports would be 4.8, 3.3, 1.7, 0.42, and 0.21 mg/m^3 for 10 min, 30 min, and 1, 4, and 8 h, respectively. These highly derivative estimates are comparable to, and supportive of, AEGL-3 estimates derived from the experimental data of Kumar and Vijayarhagavan (1998) (see Table 2–11).

8. SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity End Points

Human data a re available from several independent sources that define the exposure-response for AEGL-1 and AEGL-2 effects. Although a definitive demarcation of the exposure-response for sensitive populations was not provided by those data, the human data eliminated the uncertainties inherent in the use of data from animal studies. Both the AEGL-1 and AEGL-2 values were based on effect end points consistent with the respective AEGL definitions (i.e., threshold for barely discernible ocular irritation

[AEGL-1] and threshold for ocular irritation indicative of functional impairment [AEGL-2]). Areas of uncertainty were associated with the sensitive responders and the relationship between ocular effects and the onset of respiratory effects. Human data from which to develop AEGL-3 values were unavailable. The AEGL-3 was based on an estimated lethality threshold from studies in mice (Vijayaraghavan 1997; Kumar and Vijayaraghavan 1998). When compared with human exposure-response data and lethality estimates, the mouse lethality data were considered a defensible approach to AEGL-3 derivation. AEGL-3 values based on a human lethality estimate of 900 mg·min/m^3 (Reutter and Wade 1994; NRC 1997) were very similar to those developed using the animal data of Vijayaraghavan (1997) and Kumar and Vijayaraghavan (1998). An estimate of theoretical excess cancer risk based upon a geometric mean of inhalation slope factors developed using various data sets and procedures revealed that exposure concentrations representing a theoretical 10^{-4} lifetime risk were similar to the AEGL-3 exposure concentration values. The exposures for theoretical excess lifetime cancer risk at 10^{-5} and 10^{-6} levels would be correspondingly reduced. The use of excess cancer risk estimates in setting AEGL values is precluded by the uncertainties involved in assessing excess cancer risk following a single acute exposure of 8-h or less duration, by the relatively small population exposed in an emergency release situation, and by the potential risks associated with evacuations.

The AEGL values for sulfur mustard are summarized in Table 2–12. Extrapolation to exposure durations of less than 10 min is not recommended in the absence of careful evaluation of existing data and comparison of any derivative values with those data.

8.2. Comparison with Other Standards and Guidelines

Comparison of the draft AEGL values with other existing standards and guidelines is shown in Table 2–13. No other standards or guidelines from other agencies or programs (e.g., NIOSH, ERPG, ACGIH, MAK, MAC, OSHA) were available.

8.3. Data Adequacy and Research Needs

The AEGL-1 values are based on human data and are considered estimates for exposures that would cause no significant health effects or sensa

tions of irritation beyond minimal conjunctivitis. The ocular irritation on which the AEGL-1 and AEGL-2 values are based is the most sensitive response to sulfur mustard vapor. The AEGL-2 values provide Ct exposures that are well below those known to induce severe ocular effects in normal humans (i.e., 70–90 mg·min/m^3). AEGL-3 values provide Ct values (39–130 mg·min/m^3) that are at levels known to cause moderate to severe ocular irritation and possible respiratory tract irritation in human subjects (Anderson 1942; Guild et al. 1941) but no life-threatening effects or death. Although the overall database for acute inhalation exposure to sulfur mustard is not extensive, the AEGL values are supported by the available data.

TABLE 2–12 Summary of AEGL Values for Sulfur Mustard[a]

AEGL Level	10 min	30 min	1 h	4 h	8 h
AEGL-1[a] (Nondisabling)	0.06 ppm (0.40 mg/m^3)	0.02 ppm (0.13 mg/m^3)	0.01 ppm (0.067 mg/m^3)	0.003 ppm (0.017 mg/m^3)	0.001 ppm (0.008 mg/m^3)
AEGL-2[a] (Disabling)	0.09 ppm (0.60 mg/m^3)	0.03 ppm (0.20 mg/m^3)	0.02 ppm (0.10 mg/m^3)	0.004 ppm (0.025 mg/m^3)	0.002 ppm (0.013 mg/m^3)
AEGL-3[a] (Lethal)	0.59 ppm (3.9 mg/m^3)	0.41 ppm (2.7 mg/m^3)	0.32 ppm (2.1 mg/m^3)	0.08 ppm (0.53 mg/m^3)	0.04 ppm (0.27 mg/m^3)

[a]AEGL-1 and AEGL-2 values, and the 4- and 8-h AEGL-3 values are at or below the odor threshold for sulfur mustard.

The absence of multiple-species lethality data for acute exposures limits a thorough understanding of variability. Data providing definitive demarcation of the threshold for serious and/or irreversible effects would provide a more complete picture of responses resulting from acute inhalation exposure to sulfur mustard. That is especially relevant to assessing the potential for serious respiratory tract damage or permanent ocular pathology following acute exposure. Although sulfur mustard is a genotoxic chemical capable of inducing tumors in animals and humans, the carcinogenic potential of acute inhalation exposures has not been defined.

TABLE 2–13 Comparison of AEGL Values for Sulfur Mustard with Other Extant Standards and Guidelines

Guideline	10 min	30 min	1 h	4 h	8 h	Other
AEGL-1	0.40 mg/m^3 (0.06 ppm)	0.13 mg/m^3 (0.02 ppm)	0.067 mg/m^3 (0.01 ppm)	0.017 mg/m^3 (0.003 ppm)	0.008 mg/m^3 (0.001 ppm)	
AEGL-2	0.60 mg/m^3 (0.09 ppm)	0.20 mg/m^3 (0.03 ppm)	0.10 mg/m^3 (0.02 ppm)	0.025 mg/m^3 (0.004 ppm)	0.013 mg/m^3 (0.002 ppm)	
AEGL-3	3.9 mg/m^3 (0.59 ppm)	2.7 mg/m^3 (0.41ppm)	2.1 mg/m^3 (0.32 ppm)	0.53 mg/m^3 (0.08 ppm)	0.27 mg/m^3 0.04 ppm)	
Department of the Army/Civilian Occuptional WPL[a]					0.003 mg/m^3 (0.0005 ppm)	
Department of the Army/Civilian GPL[b]						0.0001 mg/m^3 (1.5×10^{-5} ppm)
CDC-CSEPP (Thacker, 1994)[c]						2.0 mg·min/m^3 (0.3 ppm)

[a]Worker Population Exposure Limit (DA 1991, 1997; DHHS 1988), 8-h TWA, 5 d/wk.

[b]General Population Limit (no observable effects), 24-h TWA, 7 d/wk.

[c]Recommended acute effects levels for determining emergency evacuation distances in the Chemical Stockpile Emergency Preparedness Program (CSEPP); no set exposure time.

9. REFERENCES

Anderson, J.S. 1942. The effect of mustard gas vapour on eyes under Indian hot weather conditions. CDRE Report No. 241. Chemical Defense Research Establishment (India).

Anderson, D.R., S.L.Byers, C.R.Clark, and J.A.Schlehr. 1997. Biochemical alterations in rat lung lavage fluid following acute sulfur mustard inhalation. Inhalation Toxicol. 9:43–51.

Anderson, D.R., J.J.Yourick, R.B.Moeller, J.P.Petrali, G.D.Young, and S.L. Byers. 1996. Pathologic changes in rat lungs following acute sulfur mustard inhalation. Inhalation Toxicol. 8:285–297.

ATSDR (Agency for Toxic Substances and Disease Registry). 1992. Toxicological Profile for Mustard Gas. TP-91/22. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Back, K.C., A.A.Thomas, and J.D.MacEwen. 1972. Reclassification of material listed as transportation health hazards. TSA-2072–3, PB-214270/1, Office of Hazardous Materials, Office of the Assistant Secretary for Safety and Consumer Affairs, Department of Transportation, Washington, DC.

Bakshi, K.F., S.N.J.Pang, and R.Snyder. 2000. Review of the U.S. Army's health risk assessments for oral exposure to six chemical warfare agents. J. Toxicol. Environ. Health (Part A) 59(5–6):281–526.

Beebe, G.W. 1960. Lung cancer in World War I veterans: Possible relation of mustard gas injury and 1918 influenza epidemic. J. Natl. Cancer Inst. 25:1231– 1252.

Blewett, W.K. 1986. Tactical weapons: Is mustard still king? NBC Defense Technol. Int. 1:64–66.

Bloom et al. 1944. Informal monthly progress report on toxicity of chemical warfare agents. OSRD Informal Report 9–4–1–12. January 10, 1944. (As cited in DNA 1993.)

Boursnell, J.C., J.A.Cohen, M.Dixen, et al. 1946. Studies on mustard gas (ββ'-dichlorodiethyl sulphide) and some related compounds. 5. The fate of injected mustard gas (containing radioactive sulphur) in the animal body. Biochem. J. 40:756–764.

Bowden, E. 1943. Median detectable concentrations by odor of plant run mustard, plant run Lewisite and pilot plant ethyl nitrogen mustard. ADB 969801, TDMR 615 (April 1943).

Budavari, S, M.J.O'Neil, A.Smith, and P.R.Heckelman, eds. 1989. The Merck Index: An encyclopedia of chemicals, drugs, and biologicals, 11th Ed. Rahway, NJ: Merck and Co., Inc.

Büscher, H. 1932. Green and Yellow Cross: Special Pathology and Therapy of Injuries Caused by the Chemical War Materials of The Green Cross Group (Phosgene and Diphosgene) and of The Yellow Cross Group (Mustard Gas and

Lewisite) [in German]. R.Himmelhaber and Co., Hamburg, Germany. Translated from the German in 1944 by N.Conway, Kettering Laboratory of Applied Physiology, University of Cincinnati, Cincinnati, OH.

Calvet, J.H., P.H.Jarreau, M.Levamve, M.P.D'Ortho, H.Lorino, A.Harf, I. Macquin-Mavier. 1994. Acute and chronic respiratory effects of sulfur mustard intoxication in guinea pig. J. Appl. Physiol. 76:681–688.

Case, R.A.M., and A.J.Lea. 1955. Mustard gas poisoning, chronic bronchitis and lung cancer. An investigation into the possibility that poisoning by mustard gas in the 1914–1918 war might be a factor in the production of neoplasia. Brit. J. Prev. Med. 9:62–72.

Cowan, F.M., J.J.Yourick, C.G.Hurst, C.A.Broomfield, and W.J.Smith. 1993. Sulfur mustard-increased proteolysis following in vitro and in vivo exposures. Cell Biol. Toxicol. 9:269–277.

Crump, K.S., and R.B.Howe. 1984. The multistage model with a time-dependent dose pattern: Applications to carcinogenic risk assessment. Risk Analysis 4:163–176.

D'Halluin, F., and H.Roels. 1984. Autopsy observations in an Iranian soldier exposed to war gas. pp. 284–290 in Proceedings of the First World Congress on New Compounds in Biological and Chemical Warfare: Toxicological Evaluation. State University of Ghent and the National Science Foundation of Belgium, Ghent (May 21–23, 1984).

DA (U.S. Department of the Army). 1974. Chemical agent data sheets. Vol. 1. Tech. Report E-SR-74001, Edgewood Arsenal Special Report, U.S. Department of the Army, Defense Technical Information Center, Alexandria, VA.

DA (U.S. Department of the Army). 1991. Occupational Health Guidelines for the Evaluation and Control of Occupational Exposure to Mustard Agents H, HD, and HT. Dept. of the Army, Pamphlet 40–173, Washington., DC.

DA (U.S. Department of the Army). 1996. Detailed and General Facts About Chemical Agents—TG 218. U.S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD.

DA (U.S. Department of the Army). 1997. The Army Chemical Agent Safety Program. Army Regulation AR 385–61. Headquarters, Department of the Army, Washington, DC.

DA (U.S. Department of the Army). 2001. Chemical Stockpile Disposal Program. Information Products Fact Sheets [Online]. Available at: http://www.pmcd. apgea.army.mil/CSDP.

Dabrowska, M.I., L.L Becks, J.L.Lelli, M.G.Levee, and D.B.Hinshaw. 1996. Sulfur mustard induces apoptosis and necrosis in endothelial cells. Toxicol. Appl. Pharmacol. 141:568–583.

Davison, C., R.S.Rozman, P.K.Smith. 1961. Metabolism of bis-β-chloroethyl sulfide (sulfur mustard gas). Biochem. Pharmacol. 65–74. (As cited in IARC 1975.)

DHHS (U.S. Department of Health and Human Services). 1988. Final recommendations for protecting the health and safety against potential adverse effects of long-term exposure to low doses of agents: GA, GB, VX, mustard agent (H, HD, HT), and lewisite (L). U.S. Department of Health and Human Services, Centers for Disease Control. Fed. Reg. 53 (50):8504–8507.

DHHS (U.S. Department of Health and Human Services). 2000. Ninth Report on Carcinogens. Mustard Gas. U.S. Department of Health and Human Services.

Dixon, M., and D.Needham. 1946. Biochemical research on chemical warfare agents. Nature 158:432–438.

DNA (Defense Nuclear Agency). 1993. Defense Nuclear Agency improved casualty estimation (DICE). Chemical Insult Program: Acute Chemical Agent Exposure Effects. Technical Report Prepared for Director, Defense Nuclear Agency, Washington, DC, by EAI Corporation, Abingdon, MD, under Contract No. DNA 001-C-90–0164 (16 April 1993, p. A-1) .

Dudley, H.C., and W.J.H.B.Wells. 1938. The detection of HS by odor. ADB 959500, EATR 249 (March 1936). (As cited in personal communication from S.Reutter, U.S. Army Edgewood Research Development and Engineering Center, APG, MD, October 1995.)

Dunn, P. 1986. The chemical war: Iran revisited—1986. NBC Defense Technol. Int. 1:32–39.

Easton, D.F., J.Peto, and R.Doll. 1988. Cancers of the respiratory tract in mustard gas workers. Br. J. Ind. Med. 45:652–659.

Eisenmenger, W., G.Drasch, M.von Clarmann, E.Kretschmer, and G.Roider. 1991. Clinical and morphological findings on mustard gas [bis(2-chloroethyl)sulfide] poisoning. J. Forensic Sciences 36:1688–1698.

Emad, A., and G.R.Rezaian. 1997. The diversity of the effects of sulfur mustard gas inhalation on respiratory system 10 years after a single, heavy exposure. Chest 112:734–738.

Emison, E.S., and W.J.Smith. 1997. Cytometric analysis of DNA damage in cultured human epithelial cells after exposure to sulfur mustard. J. Amer. Coll. Toxicol. 15(Suppl. 2):S9–S18.

EPA (U.S. Environmental Protection Agency). 1991. Upper-bound quantitative cancer risk estimate for populations adjacent to sulfur mustard incineration facilities. EPA/600/8–91/053. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.

Fox, M., and D.Scott. 1980. The genetic toxicology of nitrogen and sulfur mustard. Mutat Res. 75:131–168.

Fuhr, I., and E.H.Krakow. 1945. Median lethal concentrations of H for mice and rats. For various exposure times. MDR 21, March 21. (As cited in McNamara et al. 1975.)

Gross, C., H.Meier, B.Papirmeister, F.Brinkley, and J.Johnson. 1985. Sulfur mustard lowers nicotinamide adenine dinucleotide concentrations in human

skin grafted to athymic nude mice. Toxicol. Appl. Toxicol. 81:85–90.

Guild, W.J., K.P.Harrison, A.Fairly, and A.E.Childs. 1941. The effect of mustard gas vapour on the eyes. Porton Report No. 2297, Serial No. 12, 8 November 1941.

Heyndrickx, A., H.De Puydt, and J.Cordonnier, 1984. Comparative study of two different field tests for the detection of yperite in the atmosphere, applied on biological samples of gassed soldiers, pp. 61–68 in Proceedings of the First Word Congress on New Compounds in Biological and Chemical Warfare: Toxicological Evaluation. State University of Ghent and the National Science Foundation of Belgium, Ghent (May 21–23, 1984).

Henriques, F.C., Jr., A.R.Moritz, H.S.Breyfogle, and L.A.Patterson. 1943. The Mechanism of Cutaneous Injury by Mustard Gas. An Experimental Study Using Mustard Prepared with Radioactive Sulfur. Div. 9 National Defense Res. Comm. of the Office of Scientific Research and Development, Washington, D.C.. Contract No. NDRC 169, Formal Progress Report. DTIC No. ATI 30343. (As cited in Papirmeister et al. 1991.)

Heston, W.E, and W.D.Levillian. 1953. Pulmonary tumors in strain A mice exposed to mustard gas. Proc. Soc. Exp. Biol. (NY) 82:457–460. (As cited in IARC 1975.)

IARC (International Agency for Research on Cancer). 1975. IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Man: Some Aziridines, N, S, & O-mustards and Selenium, vol. 9, pp. 181–207. International Agency for Research on Cancer, Lyons, France.

IARC (International Agency for Research on Cancer). 1982. IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Chemicals, Industrial Processes and Industries Associated with Cancer in Humans. Volumes 1–29, Suppl. 4, pp. 163–164. International Agency for Research on Cancer, Lyons, France.

IARC (International Agency for Research on Cancer). 1987a. IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Man: Overall Evaluation of Carcinogenicity: An Updating of IARC Monographs Volumes 1–42, Suppl. 7, pp. 67, 259–260 and 398. International Agency for Research on Cancer, Lyons, France.

IARC (International Agency for Research on Cancer). 1987b. IARC Monograph on the Evaluation of the Carcinogenic Risks to Humans: Genetic and Related Effects: An Updating of Selected IARC Monographs Volumes 1–42, Suppl. 6, pp. 403–405. International Agency for Research on Cancer, Lyons, France.

Inada, S., K.Hiragun, K.Seo, and T.Yamura. 1978. Multiple Bowen's disease observed in former workers of a poison gas factory in Japan, with special reference to mustard gas exposure. J. Dermatol. 5:49–60.

IOM (Institute of Medicine, Committee to Survey the Health Effects of Mustard Gas and Lewisite, Division of Health Promotion and Disease Prevention).

1993. Veterans at Risk; The Health Effects of Mustard Gas and Lewisite, C.M. Pechura and D.P.Rall, eds. Washington, DC: National Academy Press.

Klehr, N. 1984. Cutaneous late manifestation in former mustard gas workers [in German]. Z.Hautkrankh. 59:1161–1170.

Kumar, O., and R.Vijayaraghavan. 1998. Effect of sulphur mustard inhalation exposure on some urinary variables in mice. J. Appl. Toxicol. 18:257–259.

Langenberg, J.P., G.P.van der Schans, H.E.T.Spruit, et al. 1998. Toxicokinetics of sulfur mustard and its DNA-adducts in the hairless guinea pig. Drug. Chem. Toxicol. 21(Suppl. 1):131–147.

Laughlin, R.C. 1944. Correlation of eye changes in rabbits with CT exposure to H. MRL (EA) Report 23. 12 June 1944.

Levy, M. 1934. The effect of mustard gas upon property of nucleated erythrocytes and their ghosts. Cancer Res. 25:752–759.

Lin, P., F.L.Vaughan, and I.A.Bernstein. 1996. Formation of interstrand DNA cross-links by bis-(2-chloroethyl)sulfide (BCES): A possible cytotoxic mechanism in rat keratinocytes. Bioch. Biophys. Res. Comm. 218:556–561.

Lohs, K. 1975. Delayed Toxic Effects of Chemical Warfare Agents: A SIPRI Monograph. Stockholm: Almqvist and Wiksell.

Mandl, H., and G.Freilinger. 1984. First report on victims of chemical warfare in the Gulf-War treated in Vienna, pp. 330–340 in Proceedings of the First World Congress on New Compounds in Biological and Chemical Warfare: Toxicological Evaluation. State University of Ghent and the National Science Foundation of Belgium, Ghent (May 21–23, 1984).

Manning, K.P., D.C.G.Skegg, and P.M Stell. 1981. Cancer of the larynx and other occupational hazards of mustard gas workers. Clin. Otolaryngol. 6:165–170.

McNamara, B.P., E.J.Owens, M.K.Christensen, F.J.Vocci, D.F.Ford, and H. Rozimarek. 1975. Toxicological basis for controlling levels of mustard in the environment. EASP EBSP 74030. Biomedical Laboratory, Department of the Army, Headquarters, Edgewood Arsenal, Aberdeen Proving Ground, MD.

Moore, A.M, and J.B.Rockman. 1950. A study of human hypersensitivity to compounds of the mustard gas type. Canadian J. Res. 28:169–176.

Opresko, D.M., R.A.Young, R.A.Faust, S.S.Talmage, A.P.Watson, R.H.Ross, K.A.Davidson, and J.King. 1998. Chemical warfare agents: Estimating oral reference doses. Rev. Environ. Contam. Toxicol. 156:1–183.

Nagy, S.M., C.Golumbic, W.H.Stein, J.S.Fruton, and M.Bergmann. 1946. The penetration of vesicant vapors into human skin. J. Physiol. 29:441–469.

Nakamura, T. 1956. Studies on the warfare gas-injury in Japan. Report I. On the general condition of the poison gas island [in Japanese]. Hiroshima Med. J. 4:1141–1149. (As cited in Inada et al. 1978.)

Nicholson, W., and Watson, A. 1993. Risk assessment considerations for sulfur mustard. Appendix I, pp 390–398 in Veterans at Risk: The Health Effects of Mustard Gas and Lewisite, C.M.Pechura and D.P.Rall, eds. Washington, DC: National Academy Press.

Nishimoto, Y., M.Yamakido, T.Shigenobu, K.Onari, and M.Yukutake. 1983. Long term observations of poison gas workers with special reference to respiratory cancers. J. Univ. Occup. Environ. Health 5(suppl):89–94.

Nishimoto, Y., M.Yamakido, and Ishioka et al. 1988. Epidemiological studies of lung cancer in Japanese mustard gas workers. In: Unusual Occurrences as Clues to Cancer Etiology, R.W.Miller et al., eds. pp. 95–101. Tokyo: Japan Sci. Soc. Press.

Norman, J. 1975. Lung cancer mortality in World War I veterans with mustard gas injury: 1919–1945. J. Natl. Cancer Inst. 54:311–317.

NRC (National Research Council). 1985. Emergency and continuous exposure guidance levels for selected airborne contaminants, Vol. 5. Washington, DC: National Academy Press.

NRC (National Research Council). 1986. Criteria and methods for preparing emergency exposure guidance level (EEGL), short-term public emergency guidance level (SPEGL), and continuous exposure guidance level (CEGL) documents. Washington, DC: National Academy Press. Appendix F, pp. 25–27.

NRC (National Research Council). 1997. Review of acute human-toxicity estimates for selected chemical-warfare agents. Washington, DC: National Academy Press.

NRC (National Research Council). 1999. Review of the U.S. Army's Health Risk Assessments for Oral Exposure to Six Chemical Warfare Agents. Washington, DC: National Academy Press.

Papirmeister, B., C.Gross, H.Meier, J.Petrali, and J.Johnson. 1985. Molecular basis for mustard-induced vesication. Fundam. Appl. Toxicol. 5:S134–S149.

Papirmeister, B., A.J.Feister, S.I.Robinson, and R.D.Ford. 1991. Medical Defense Against Mustard Gas: Toxic Mechanisms and Pharmacological Implications. Boca Raton, FL: CRC Press.

Petrali, J.P., and S.Oglesby-Megee. 1997. Toxicity of mustard gas lesions. Microscopy Res. and Tech. 37:221–228.

Reed, C.I. 1918. The minimum concentration of mustard gas effective for man. Preliminary Report. Report 318. War Department, Med. Div., C.W.S. Pharmacol. Res. Sec. Amer. Univ. Exp. Station, War Dept. October 26, 1918.

Reed, C.I., E.F.Hopkins, and C.F.Weyand. 1918. The minimum concentration of mustard gas effective for man. Final Report. Report 329. War Department, Med. Div., C.W.S., Pharmacol. Res. Sec. Amer. Univ. Exp. Station, War Dept. December 2, 1918.

Renshaw, B. 1947. Observations on the role of water in the susceptibility of human skin to injury by vesicant vapors. J. Invest. Dermatology. 9:75–85.

Reutter, S.A., and J.V.Wade. 1994. "Table 1; Summary of Existing and Recommended Estimates (U)," unclassified summary table. In: Review of existing toxicity data and human estimates for selected chemical agents and

recommended human toxicity estimates appropriate for defending the soldier. ERDEC-SP-018. U.S. Department of the Army, Edgewood Research Development and Engineering Center, Aberdeen Proving Ground, MD (secret report).

Robinson, J.P. 1967. Chemical warfare. Science J. 4:33–40.

Rosenblatt, D.H., T.A.Miller, J.C.Dacre, I.Muul, and D.R.Cogley. 1987. Problem definition studies on potential environmental pollutants. II. Physical, chemical, toxicological, and biological properties of 16 substances. U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, MD. Technical Report 7509.

Rosenblatt, D.H. 1987. Recalculation of general population exposure limits and stack detection levels for mustard (unpublished). U.S. Army Biomedical Research and Development Laboratory, Fort Derrick, MD

Ruth, J.H. 1986. Odor Thresholds and Irritation Levels of Several Chemical Substances: A review. Am. Ind. Hyg. Assoc. J. 47:A142–A151.

Shakil, F.A., M.Kuramoto, Yamakido et al. 1993. Cytogenetic abnormalities of hematopoietic tissue in retired workers of the Ohkunojima poison gas factory. Hiroshima J. Med. Sci. 42:159–165.

Smith, W.J., C.L.Gross, P.Chan, and H.L.Meier. 1990. The use of human epidermal keratinocytes in culture as a model for studying the biochemical mechanisms of sulfur mustard induced vesication. Cell Biol. Toxicol. 6:285–291.

Smith, E.S, and W.J.Smith. 1997. Cytometric analysis of DNA damage in cultured human epithelial cells after exposure to sulfur mustard . J. Amer. Coll. Toxicol. 15(Suppl. 2):S9–S18.

Sulzberger, M.B., R.L.Bauer, A.Kanof, and C.Lowenberg. 1945. Skin sensitization to vesicant agents of chemical warfare. Fasciculus on Chemical Warfare Medicine 3:16–66.

Takeshima, Y., K.Inai, W.P.Bennet et al. 1994. P53 mutations in lung cancers from Japanese mustard gas workers. Carcinogenesis 15:2075–2079.

ten Berge, W.F., A.Zwart, and L.M.Appelman. 1986. Concentration-time mortality response relationship of irritant and systemically acting vapours and gases. J. Hazard Materials 13:301–309.

Thacker, S.B., Assistant Surgeon General, Acting Director, National Center for Environmental Health, Centers for Disease Control and Protection, U.S. Department of Health and Human Services. 1994. Letter establishing "Recommended Acute Threshold Effects Levels for Determining Emergency Evacuation Distances in the CSEPP Program," to Col. J.M.Coverstone, Deputy for Chemical Demilitarization, Office of the Assistant Secretary, (I, L & E), The Pentagon, Washington, DC (24 June 1994).

U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM). 2000. Evaluation of Airborne Exposure Limits for Sulfur Mustard: Occupational and General Population Exposure Criteria. Aberdeen Proving Ground, Maryland. Technical Report 47-EM-3767–00.

Vijayaraghavan, R. 1997. Modifications of breathing pattern induced by inhaled sulphur mustard in mice. Arch. Toxicol. 71:157–164.

Vogt, R., A.Dannenberg, B.Scholfield, N.Hynes, and B.Papirmeister. 1984. Pathogenesis of skin lesions caused by sulfur mustard. Fundam. Appl. Toxicol. 4:571–583.

Wada, S., Y.Nishimoto, M.Miyanishi et al., 1968. Mustard gas as a cause of respiratory neoplasm in man. The Lancet, June 1, 1968, pp. 1161–1163.

Walker, H.W., B.F.Smith, and A.Blake. 1928. Summary and Discussion of Available Mustard Gas Data from Field Tests Conducted Prior to 1928. Report. No. 462, Chemical Warfare Service, Edgewood, Arsenal, Edgewood, MD.

Warthin, A.S., and C.V.Weller. 1919. The lesions of the respiratory and gastrointestinal tracts produced by mustard gas (dichloroethyl sulphide). J. Lab. Clin. Med. 4:229–264.

Waters, M.D., N.E.Garrett, C.M.Covon de Serres, B.E.Howard, and H.F.Stack. 1983. Genetic toxicology of some known or suspected human carcinogens, Vol. 8. Pp. 261–341 In: Chemical Mutagens, Principles and Methods for their Detection, F.J.de Serres, ed. New York, NY: Plenum Press.

Watson, A.P., T.D.Jones, and G.D.Griffin. 1989. Sulfur mustard as a carcinogen: Application of relative potency analysis to the chemical warfare agents H, HD, and HT . Reg. Toxicol. Pharmacol. 10:1–25.

Watson, A.P., and G.D.Griffin. 1992. Toxicity of vesicant agents scheduled for destruction by the chemical stockpile disposal program. Environ. Health Perspect. 98:259–280.

Weiss, A., and B.Weiss. 1975. Karzinogenese durch Lost-Exposition beim Menschen, ein wichtiger Hinweis fur die alkylantien-Therapie. Dtsch. Med. Wschr. 100:919–923. (As cited in IARC 1975.)

Wulf, H.C., A.Aastad, E.Darre, and E.Niebuhr. 1985. Sister chromatid exchanges in fishermen exposed to occupational mustard gas poisoning. The Lancet, March 25, 1985, pp. 690–691.

Yamada, A., F.Hirose, and M.Miyanishi. 1953. An autopsy of bronchial carcinoma found in a patient succumbed to occupational mustard gas poisoning. Gann 44:216–219. (As cited in IARC 1975.)

Yamada, A., F.Hirose, M.Nagia, and T.Nakamura. 1957. Five cases of cancer of the larynx found in persons who suffered from occupational mustard gas poisoning. Gann 48:366–368. (As cited in IARC 1975.)

Yamada, A. 1963. On the late injuries following occupational inhalation of mustard gas, with special references to carcinoma of the respiratory tract. Acta Pathologica Japonica. 13:131–155.

Yamada, A. 1974. Patho-anatomical studies on occupational poisoning. Tr. Soc. Path. Jap. 63:17–61. (As cited in Inada et al. 1978.)

Yamakido, M., Y.Nishimoto, T.Shigenobu, K.Onari, C.Satoh, K.Goriki, and M.

Fujita. 1985. Study of genetic effects of sulphur mustard gas on former workers of Okunojima poison gas factory and their offspring. Hiroshima J. Med. Sci. 34:311–322.

Yamakido, M., S.Ishioka, K.Hiyama, and A.Meado. 1996. Former poison gas workers and cancer: Incidence and inhibition of tumor formation by treatment with biological response modifier N-CWS. Environ. Health Perspect. 104:485– 488.

Yanagida, J., S.Hozawa, M.Edson, and E.Estok. 1988. Somatic mutation in peripheral lymphocytes of former workers at the Okunojima poison gas factory. Jap. J. Cancer Res. 79:1276–1283.

Appendixes

APPENDIX A

Derivations of AEGL Values

Derivation of AEGL-1

Key study:	Anderson (1942)
Toxicity end point:	Exposure concentration-time product of 12 mg· min/m^3 represented the threshold for ocular effects (conjunctival injection and minor discomfort with no functional decrement) for human volunteers exposed to agent HD at varying exposure regimens. The eye is generally considered to be the most sensitive organ/ tissue relative to agent HD exposure.
Scaling:	The concentration-time relationship for many irritant and systemically acting vapors and gases can be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al. 1986). Analysis of available data indicated n to be near unity (Appendix B), hence, $C^1 \times t = k$.
Uncertainty factors:	Total adjustment of 3. A factor of 3 was applied for intraspecies variability (protection of sensitive populations). This factor was limited to 3 under the assumption that the primary mechanism of action of agent HD involves a direct effect on the ocular surface and that the response will not vary greatly among individuals. In addition, subjects in the Anderson (1942) study exhibited little variability in ocular response. Because the AEGL-1 is based on human data, the interspecies UF is 1.

10-min AEGL-1:	$C^1 \times 10$ min=12 mg·min/m^3 C=1.2 mg/m^3 10-min AEGL-1=(1.2 mg/m^3)/3=0.40 mg/m^3 (0.06 ppm)
30-min AEGL-1:	$C^1 \times 30$ min=12 mg·min/m3 C=0.4 mg/m^3 30-min AEGL-1=(0.4 mg/m^3)/3=0.13 mg/m^3 (0.02 ppm)
1-h AEGL-1:	$C^1 \times 60$ min=12 mg·min/m^3 C=0.2 mg/m^3 1-h AEGL-1=(0.2 mg/m^3)/3=0.067 mg/m^3 (0.01 ppm)
4-h AEGL-1:	$C^1 \times 240$ min=12 mg·min/m3 C=0.05 mg/m^3 4-h AEGL-1=(0.05 mg/m^3)/3=0.017 mg/m^3 (0.003 ppm)
8-h AEGL-1:	$C^1 \times 480$ min=12 mg·min/m3 C=0.025 mg/m^3 8-h AEGL-1=(0.025 mg/m^3)/3=0.008 mg/m^3 (0.001 ppm)

Derivation of AEGL-2

Key study:	Anderson (1942)
Toxicity end point:	A concentration-time product of 60 mg·min/m^3 was considered the lowest exposure causing ocular effects (well-marked, generalized conjunctivitis, edema, photophobia, and irritation) resulting in effective performance decrement and characterized as a military casualty requiring treatment for up to 1 wk.

Scaling:	The concentration-time relationship for many irritant and systemically acting vapors and gases may be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al. 1986). Analysis of available data indicated n to be near unity (Appendix B), hence, $C^1 \times t = k$.
Uncertainty factors:	Total adjustment of 10. A factor of 3 was applied for intraspecies variability (protection of sensitive populations). This factor was limited to 3 under the assumption that the primary mechanism of action of agent HD involves a direct effect on the ocular surface and that this response will not vary greatly among individuals. Because the AEGL-1 is based on human data, the interspecies UF is 1. A modifying factor of 3 was applied to accommodate potential onset of long-term ocular or respiratory effects. Because the factors of 3 each represent a logarithmic mean (3.16) of 10, their product is 3.16×3.16=10.
10-min AEGL-2:	$C^1 \times 10$ min=60 mg·min/m3 C=6 mg 10-min AEGL-2=(6 mg/m^3)/10=0.60 mg/m^3 (0.09 ppm)
30-min AEGL-2:	$C^1 \times 30$ min=60 mg·min/m3 C=2.00 mg 30-min AEGL-2=(2.00 mg/m^3)/10=0.20 mg/m^3 (0.03 ppm)
1-h AEGL-2:	$C^1 \times 60$ min=60 mg·min/m3 C=1.00 mg/m^3 1-h AEGL-2=(1.00 mg/m^3)/10=0.10 (0.02 ppm)
4-h AEGL-2:	$C^1 \times 240$ min=60 mg·min/m3

	C=0.25 mg/m^3 4-h AEGL-2=(0.25 mg/m^3)/10=0.025 mg/m^3 (0.004 ppm)
8-h AEGL-2:	C^1×480 min=60 mg·min/m3 C=0.125 mg/m^3 8-h AEGL-2=(0.125 mg/m^3)/10=0.013 mg/m^3 (0.002 ppm)

Derivation of AEGL-3

Key study:	Kumar and Vijayaraghavan (1998)
Toxicity end point:	Estimated lethality threshold of 21.2 mg/m^3 for 1 h based on no deaths in mice exposed to that concentration, which is 0.5 of the 1-h LC_{50} in mice reported by Vijayaraghavan (1997).
Scaling:	The concentration-time relationship for many irritant and systemically acting vapors and gases may be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al. 1986). Analysis of available data pertaining to ocular effects indicated n to be near unity (Appendix B). However, there was uncertainty regarding the validity of applying linear extrapolation based on ocular effects to concentration-time extrapolations for lethality. Therefore, in the absence of chemical-specific lethality data, time scaling was performed using exponential extrapolation (n =3) for shorter time periods (<1 h) and linear extrapolation (n=1) for longer time periods (>1 h), thereby providing a somewhat more conservative (i.e., protective) estimate of the AEGL-3 values than would be obtained using an n value based on ocular irritation. The concentration-time constant, k, was 1,272 mg·min/m^3 where n=1 and 571,687.68 mg·min/m^3

	where n=3.
Uncertainty factors:	Total UF was 10. A UF for interspecies was limited to 3 because human data are available showing that exposures to the AEGL-3 values are more likely to produce only severe ocular irritation and possible minor or moderate irritation of the upper respiratory tract. Intraspecies variability was limited to 3 because lethality appears to be a function of extreme pulmonary damage resulting from direct contact of the agent with epithelial surfaces. No modifying factor was applied because the basis of lethality estimate was from a studies utilizing a 14-d observation period to assess the lethal response from a 1-h exposure. Because the factors of 3 each represent a logarithmic mean (3.16) of 10, their product is 3.16×3.16=10.
10-min AEGL-3:	C^3×10 min=571,687.68 mg·min/m^3 C^3=57,168.76 mg·min/m3 C=38.52 mg/m^3 10-min AEGL-3=(38.52 mg/m^3)/10=3.9 mg/m^3 (0.59 ppm)
30-min AEGL-3:	C^3×30 min=571,687.68 mg·min/m3 C^3=19,056.26 mg·min/m3 C=26.7 mg/m^3 30-min AEGL-3=(26.7 mg/m^3)/10=2.7 mg/m^3 (0.41 ppm)
1-h AEGL-3:	C^1×60 min=1,272 mg·min/m3 C=21.2 mg/m^3 1-h AEGL-3=(21.2 mg/m^3)/10=2.1 mg/m^3 (0.32 ppm)
4-h AEGL-3:	C^1×240 min=1,272 mg·min/m^3 C=5.3 mg/m^3

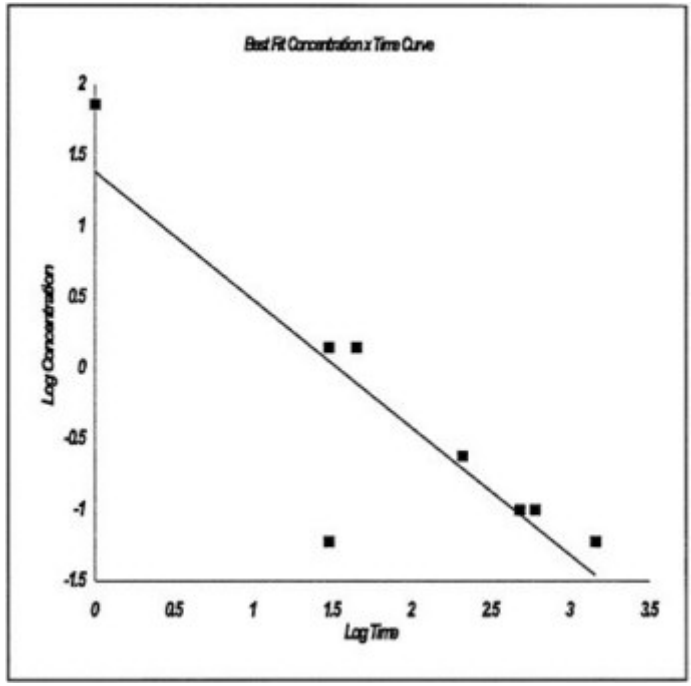
	4-h AEGL-3=(5.3 mg/m^3)/10=0.53 mg/m^3 (0.08 ppm)
8-h AEGL-3:	C^1×480 min=1,272 mg·min/m3 C=2.65 mg/m^3 8-h AEGL-3=(2.65 mg/m^3)/10=0.27 mg/m^3 (0.04 ppm)

APPENDIX B

Determination of Temporal Scaling Factor (n) for AEGL Derivations

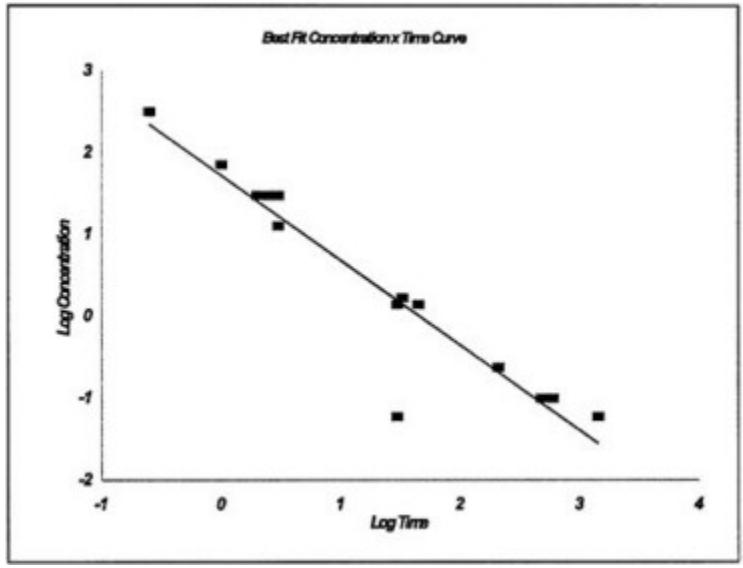
Derivation of n for $C^n \times t = k$; data points indicative of a 100% response for mild ocular irritation following exposure to sulfur mustard (agent HD) at various concentrations and times (Reed 1918; Reed et al. 1918; Guild et al. 1941; Anderson 1942)

Time	Concentration	Log Time	Log Concentration
1	72	0.0000	1.8573
30	1.4	1.4771	0.1461
30	0.06	1.4771	−1.2218
45	1.4	1.6532	0.1461
210	0.24	2.3222	−0.6198
480	0.1	2.6812	−1.0000
600	0.1	2.7782	−1.0000
1,440	0.06	3.1584	−1.2218
Regression output:			
Intercept			1.3852
Slope			−0.9002
R squared			0.7434
Correlation			−0.8622
Degrees of freedom			6
Observations			8
n=1.11			
k=34.58			



Derivation of n for $C^n \times t = k$; data points indicative of a 75–100% response for mild ocular irritation following exposure to sulfur mustard (agent HD) at various concentrations and times (Reed 1918; Reed et al. 1918; Guild et al. 1941; Anderson 1942)

Time	Concentration	Log Time	Log Concentration
1	72	0.0000	1.8573
30	1.4	1.4771	0.1461
30	0.06	1.4771	−1.2218
45	1.4	1.6532	0.1461
210	0.24	2.3222	−0.6198
480	0.1	2.6812	−1.0000
600	0.1	2.7782	−1.0000
1,440	0.06	3.1584	−1.2218
33	1.7	1.5185	0.2304
3	12.7	0.4771	1.1038
3	30	0.4771	1.4771
2.5	30	0.3979	1.4771
2	30	0.3010	1.4771
0.25	320	−0.6021	2.5051
Regression output:			
Intercept			1.7240
Slope			−1.0356
R squared			0.8891
Correlation			−0.9429
Degrees of freedom			12
Observations			14
n=0.96			
k=46.05			



APPENDIX C

Carcinogenicity Assessment for Acute Exposure to Sulfur Mustard (Agent HD)

The cancer assessment for acute inhalation exposure to sulfur mustard was conducted following the NRC methodology for EEGLs, SPEGLs, and CEGLs (NRC 1986). The virtually safe dose (VSD) was determined from an inhalation slope factor of 14 $(mg/kg/d)^{-1}$ for the general population (USACHPPM 2000). The slope factor was a geometric mean of slope factors developed using various data sets and procedures and was considered the most tenable quantitative assessment for potential cancer risk from inhalation exposure to sulfur mustard. The corresponding Inhalation Unit Risk was 0.0041 $(\mu g/m^3)^{-1}$ or 4.1 $(mg/m^3)^{-1}$ (USACHPPM 2000). The VSD was calculated as follows:

VSD=Risk Level/Unit Risk

$$VSD = \frac{1 \times 10^{-4}\ risk}{(4.1\ mg/m^3)^{-1}} = 2.5 \times 10^{-5}\ mg/m^3$$

Assuming the carcinogenic effect to be a linear function of cumulative dose *(d),* a single-day exposure is equivalent to $d \times 25{,}600$ d (average lifetime).

24-h exposure=VSD×25,600
$=(2.5\times10^{-5}\ mg/m^3)\times25{,}600$
$=0.64\ mg/m^3$

Adjustment to allow for uncertainties in assessing potential cancer risks under short term exposures under the multistage model (Crump and Howe 1984).

$$\frac{24-hr\ exposure}{6} = \frac{0.64\ mg/m^3}{6} = 0.1\ mg/m^3$$

If the exposure is limited to a fraction *(f)* of a 24-h period, the fractional exposure becomes $1/f \times 24$ h (NRC 1985). For a 1×10^{-4}, 1×10^{-5}, and 1×10^{-6} risk, the fractional exposures are shown below.

Exposure Duration	10^{-4}	10^{-5}	10^{-6}
24-h	0.1 mg/m^3 (0.02 ppm)	0.01 mg/m^3 (0.002 ppm)	0.001 mg/m^3 (0.002 ppm)
8-h	0.3 mg/m^3 (0.05 ppm)	0.03 mg/m^3 (0.005 ppm)	0.003 mg/m^3 (0.0005 ppm)
4-h	0.6 mg/m^3 (0.09 ppm)	0.06 mg/m^3 (0.009 ppm)	0.006 mg/m^3 (0.0009 ppm)
1-h	2.4 mg/m^3 (0.36 ppm)	0.24 mg/m^3 (0.036 ppm)	0.024 mg/m^3 (0.0036 ppm)
30-min	4.8 mg/m^3 (0.72 ppm)	0.48 mg/m^3 (0.072 ppm)	0.048 mg/m^3 (0.0072 ppm)
10-min	14.1 mg/m^3 (2.16 ppm)	1.41 mg/m^3 (0.22 ppm)	0.141 mg/m^3 (0.022 ppm)

Because the derivation of the cancer slope factor requires conversion of animal doses to human equivalent doses, no reduction of exposure levels is applied to account for interspecies variability. With the exception of the 10-min, 30-min, and 1-h values for 10^{-4} risk and the 10-min 10^{-5} risk, these exposures are at or below the odor threshold for sulfur mustard. A cancer risk assessment based on a geometric mean of inhalation slope factors developed using various data sets and procedures indicated an excess cancer risk of 1 in 10,000 (10^{-4}) may be associated with exposures similar to the AEGL-3 values. The use of excess cancer risk estimates in setting AEGL values is precluded by the uncertainties involved in assessing excess cancer risk following a single acute exposure of 8-h or less duration, by the relatively small population exposed in an emergency release situation, and by the potential risks associated with evacuations.

APPENDIX D

Sulfur Mustard (CAS NO. 505–60–2)

AEGL-1				
10 min	30 min	1 h	4 h	8 h
0.40 mg/m^3 (0.06 ppm)	0.13 mg/m^3 (0.02 ppm)	0.067 mg/m^3 (0.01 ppm)	0.017 mg/m^3 (0.003 ppm)	0.008 mg/m^3 (0.001 ppm)

Key reference: Anderson, J.S. 1942. The effect of mustard gas vapour on eyes under Indian hot weather conditions. CDRE Report No. 241. Chemical Defense Research Establishment (India)

Test species/strain/gender/number: 3–4 human volunteers

Exposure route/concentrations/durations: Vapor exposure to varying concentrations (1.7–15.6 mg/m^3) for varying durations (2–33 min)

Effects: Mild ocular effects (mild injection to notable conjunctivitis)

End point/concentration/rationale: Concentration-time threshold of 12 mg·min/m^3 for ocular effects (conjunctival injection with minor discomfort and no functional decrement)

Uncertainty factors/rationale:

Interspecies: 1 (human subjects)

Intraspecies: A factor of 3 was applied for intraspecies variability (protection of sensitive populations). This factor was limited to 3 under the assumption that the primary mechanism of action of agent HD involves a direct effect on the ocular surface and that the response will not vary greatly among individuals. Furthermore, little variability was observed in the tested subjects regarding ocular responses.

Modifying factor: None applied

Animal to human dosimetric adjustment: Not applicable

Time Scaling: $C^n \times t = k$, where n=1 based on analysis of available human exposure data for ocular effects.

Data adequacy: The key study was conducted using human volunteers thus avoiding uncertainties associated with animal studies. Ocular irritation is considered the most sensitive end point for assessing the effects of acute exposure

to sulfur mustard and the available data were sufficient for developing AEGL-1 values.

AEGL-2

10 min	30 min	1 h	4 h	8 h
0.60 mg/m^3 (0.09 ppm)	0.20 mg/m^3 (0.03 ppm)	0.10 mg/m^3 (0.02 ppm)	0.025 mg/m^3 (0.004 ppm)	0.013 mg/m^3 (0.002 ppm)

Key reference: Anderson, J.S. 1942. The effect of mustard gas vapour on eyes under Indian hot weather conditions. CDRE Report No. 241. Chemical Defense Research Establishment (India).

Test species/strain/gender/number: 3–4 human volunteers

Exposure route/concentrations/durations: Vapor exposure to varying concentrations (1.7–15.6 mg/m^3) for varying durations (2–33 min)

Effects: Ocular effects ranging from mild injection to notable conjunctivitis, photophobia, lacrimation, blepharospasm

End point/concentration/rationale: Exposure-concentration time product of 60 mg·min/m^3 representing exposure at which ocular irritation (well-marked, generalized conjunctivitis, edema, photophobia, and irritation) will occur resulting in performance decrement and necessitating medical treatment

Uncertainty factors/rationale:

Interspecies: 1 (human subjects)

Intraspecies: A factor of 3 was applied for intraspecies variability (protection of sensitive populations). This factor was limited to 3 under the assumption that the primary mechanism of action of agent HD involves a direct effect on the ocular surface and that this response will not vary greatly among individuals. Furthermore, little variability was observed in the tested subjects regarding ocular responses.

Modifying factor: A modifying factor of 3 was applied to accommodate uncertainties regarding the onset of potential long-term ocular effects or respiratory effects

Animal to human dosimetric adjustment: Not applicable

Time scaling: $C^n \times t = k$, where $n=1$ based on analysis of available human exposure data for ocular effects

Data adequacy: The key study was conducted using human volunteers, thus avoiding uncertainties associated with animal studies. The AEGL-2 values are based on ocular effects that may be considered severe enough to impair vision. The data were considered sufficient for developing AEGL-2 values.

AEGL-3

10 min	30 min	1 h	4 h	8 h
3.9 mg/m^3 (0.59 ppm)	2.7 mg/m^3 (0.41 ppm)	2.1 mg/m^3 (0.32 ppm)	0.53 mg/m^3 (0.08 ppm)	0.27 mg/m^3 (0.04 ppm)

Key reference: Kumar, O., and R.Vijayaraghavan. 1998. Effect of sulphur mustard inhalation exposure on some urinary variables in mice. J. Appl. Toxicol. 18:257–259.

Test species/strain/gender/number: Swiss mice/female/4 per exposure group

Exposure route/concentrations/durations: Head-only inhalation exposure for 1 h to sulfur mustard (>99% purity) at 21.2, 42.3, or 84.6 mg/m^3 (equivalent to 0.5, 1.0, and 2.0 LC_{50}). Subjects were sacrificed at 6, 24, or 48 h or 7 d after exposure. Three groups of 10 mice were exposed at each concentration and observed for up to 14 d.

Effects: Lethality assessed up to 14 d postexposure

End point/concentration/rationale: No mortality in mice at 14 d following 1-h exposure at 21.2 mg/m^3. The exposure was considered an estimate of the lethality threshold in mice.

Uncertainty factors/rationale:

Total uncertainty factor: 10

Interspecies: An uncertainty factor of 3 was applied to account for possible interspecies variability in the lethal response to sulfur mustard. Application of any additional uncertainty factors or modifying factors was not warranted because the AEGL-3 values are equivalent to exposures in humans that are known to produce only ocular and respiratory tract irritation.

Intraspecies: Intraspecies variability was limited to 3 because lethality appears to be a function of extreme pulmonary damage resulting from direct contact of the agent with epithelial surfaces.

Modifying factor: No modifying factor was applied because the basis of lethality estimate was from a study utilizing a 14-d observation period to assess the lethal response from a 1-h exposure

Animal to human dosimetric adjustment: Insufficient data

Time scaling: $C^n \times t = k$, where n=1 or 3. The concentration-time relationship for many irritant and systemically acting vapors and gases can be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5 (ten Berge et al. 1986). In the absence of chemical-specific lethality data, time scaling was performed using exponential extrapolation (n=3) for shorter time periods and linear extrapolation (n=1) for longer time periods, thereby providing a somewhat more conservative (i.e., protective) estimate of the

AEGL-3 values than would be obtained using an *n* value of 1 based on ocular irritation.

Data adequacy: Uncertainties exist regarding a definitive lethality threshold for single acute exposures to sulfur mustard. However, the key study appeared to be well-designed and properly conducted and is considered sufficient for developing AEGL-3 values.

3

Methyl Isocyanate[1]

SUMMARY

Methyl isocyanate (MIC) is one of the most reactive of all isocyanates and is rapidly degraded in aqueous medium (Varma and Guest 1993). Because of its reactivity, MIC is used as an intermediate in the synthesis of *N*-methylcarbamate and *N*-methylurea insecticides and herbicides (Hartung 1994). During the night of December 2–3, 1984, an estimated 30 tons of

[1]This document was prepared by the AEGL Development Team comprising Carol Forsyth (Oak Ridge National Laboratory) and National Advisory Committee (NAC) on Acute Exposure Guideline Levels for Hazardous Substances member Loren Koller (Chemical Manager). The NAC reviewed and revised the document and AEGL values as deemed necessary. Both the document and the AEGL values were then reviewed by the National Research Council (NRC) Subcommittee on Acute Exposure Guideline Levels. The NRC subcommittee concludes that the AEGLs developed in this document are scientifically valid on the basis of data reviewed by the NRC and are consistent with the NRC guidelines reports (NRC 1993, 2001).

MIC were released from a chemical plant in Bhopal, India, resulting in one of the worst industrial accidents in history (Karlsson et al. 1985).

Signs of severe irritation to the respiratory tract were reported for victims of the Bhopal disaster, and autopsies revealed the cause of death to be acute pulmonary edema (Weill 1988). Long-term pulmonary and ocular sequelae have been documented in survivors. The spontaneous abortion rate (Arbuckle and Sever 1998) and the infant death rate (Varma 1987) among women who were pregnant at the time of the release were significantly increased in the months following the disaster. Numerous animal studies corroborate the epidemiological findings in humans. A compilation of case reports in industrial workers consistently noted skin and respiratory irritation in MIC-exposed workers but no definitive case of sensitization (Ketcham 1973). The mechanism of action for the pulmonary, skin, and ocular toxicity is irritation, but the mechanism of action for the systemic effects is unknown.

AEGL-1 values were not derived. Although human and animal data were available for irritation levels, the irritation threshold for MIC may be above the level of concern for systemic effects. Experimental studies in humans indicate that both duration of exposure and concentration of MIC contribute to the severity of irritation. However, extrapolation from the short experimental durations to the longer AEGL time points may not be predictive of adverse health effects. It is not known at what concentration the risk for systemic effects, other than pulmonary edema, becomes a concern. The concentrations causing irritation in humans after several minutes (1–4 parts per million [ppm]) are similar to, or higher than, the concentrations resulting in embryo and fetal lethality in well conducted animal studies. Therefore, the results of controlled human exposures were not used in derivation of AEGL-1. However, it should be noted that exposures to MIC at concentrations below those used to calculate AEGL-1 might be associated with systemic toxicity.

Systemic toxicity data from rats and mice were used for derivation of AEGL-2. An increase in cardiac arrhythmias occurred in rats 4 months (mo) after a 2-hour (h) exposure to 3 ppm (Tepper et al. 1987). Pregnant Swiss-Webster mice were exposed to analytically monitored concentrations of MIC at 0, 2, 6, 9, and 15 ppm for 3 h on gestation day 8 (Varma 1987). Placental weights and fetal body weights were significantly reduced at all concentrations. Exposures to concentrations at 9 ppm and 15 ppm resulted in deaths of two dams in each group, a significant increase in complete litter resorption among surviving dams, and fetuses with significant reduc

tions in the lengths of the mandible and long bones. The single exposure concentration of 2 ppm for 3 h was an experimentally derived LOAEL for reduced fetal body weights in the absence of maternal toxicity. Values scaled for the derivation of the 10- and 30-minute (min), and 1-, 4-, and 8-h time points were calculated from the equation $C^n \times t = k$ where n=1. The value of n was empirically derived from regression analysis of lethality data for rats. Identical AEGL-2 values are derived based on the exposures of 3 ppm for 2 h and 2 ppm for 3 h. The experimental concentrations were reduced by a factor of 3 to estimate a threshold for effects on cardiac arrhythmias or fetal body weights. A total uncertainty factor (UF) of 30 was applied, including 3 for interspecies variation because similar developmental toxicity results have been obtained in both rats and mice and 10 for intraspecies variation because the mechanism of action for developmental toxicity is unknown.

The neonatal survival study with mice conducted by Schwetz et al. (1987) was used for derivation of AEGL-3 values. Pregnant mice were exposed to MIC at 0, 1, or 3 ppm for 6 h/day (d) on gestation days 14–17. Dams were allowed to litter for evaluation of neonatal survival. No maternal toxicity was observed at either exposure concentration. A concentration-related increase in the number of dead fetuses at birth was observed in both MIC exposure groups, and an increase in neonatal mortality during lactation was observed in the 3-ppm group. No differences in neonatal body-weight gain occurred during lactation between the treated and control groups. The 6-h exposure to 1 ppm was used to derive AEGL-3 values and is considered a NOEL for pup survival during lactation. Values scaled for the derivation of the 10- and 30-min, and 1-, 4-, and 8-h time points were calculated from the equation $C^n \times t = k$ where n=1. The value of n was empirically derived from regression analysis of lethality data for rats. A total UF of 30 was applied, including 3 for interspecies variation because similar developmental toxicity results have been obtained in both rats and mice and 10 for intraspecies variation because the mechanism of action for developmental toxicity is unknown. According to Section 2.7 of the standing operating procedures (NRC 2001), 10-min values are not to be scaled from an experimental exposure time of $\geq$4 h. However, because n was derived from exposures ranging from 7.5 min to 4 h, extrapolation from 6 h to the 10-min AEGL-3 value is valid in this instance.

The proposed values for the three AEGL classifications for the five time periods are listed in the table below.

TABLE 3–1 Summary of AEGL Values for Methyl Isocyanate

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1[a] (Non-disabling)	NR	NR	NR	NR	NR	
AEGL-2 (Disabling)	0.40 ppm (0.94 mg/m^3)	0.13 ppm (0.32 mg/m^3)	0.067 ppm (0.16 mg/m^3)	0.017 ppm (0.034 mg/m^3)	0.008 ppm (0.02 mg/m^3)	Decreased fetal body weights (Varma 1987); cardiac arrhythmias (Tepper et al. 1987)
AEGL-3 (Lethal)	1.2 ppm (2.8 mg/m^3)	0.40 ppm (0.95 mg/m^3)	0.20 ppm (0.47 mg/m^3)	0.05 ppm (0.12 mg/m^3)	0.025 ppm (0.06 mg/m^3)	Decreased pup survival during lactation (Schwetz et al. 1987)

[a]Exposure to MIC at concentrations below those used to calculate AEGL-1 may be associated with systemic toxicity.
Abbreviations: NR, not recommended.

1. INTRODUCTION

Methyl isocyanate (MIC) is one of the most reactive of all isocyanates and is rapidly degraded in aqueous medium (Varma and Guest 1993). Because of its reactivity, MIC is used as an intermediate in the synthesis of *N*-methylcarbamate and *N*-methylurea insecticides and herbicides (Hartung 1994).

During the night of December 2–3, 1984, a release occurred in a chemical plant in Bhopal, India, where MIC was used as an intermediate in the production of carbamates. An estimated 30 tons of MIC were released resulting in one of the worst industrial accidents in history (Karlsson et al. 1985). Signs of severe respiratory tract irritation were reported for victims of the Bhopal disaster and autopsies revealed the cause of death to be pulmonary edema (Varma and Guest 1993). Long-term pulmonary and ocular disease have been documented in survivors (Andersson et al. 1990).

TABLE 3–2 Chemical and Physical Data for Methyl Isocyanate

Parameter	Value	Reference
Synonyms	Isocyanatomethane; isocyanic acid methyl ester; MIC	Budavari et al. 1996
Chemical formula	C_2H_3NO	Budavari et al. 1996
Molecular weight	57.05	Budavari et al. 1996
CAS Registry Number	624–83–9	
Physical description	Liquid	Budavari et al. 1996
Vapor pressure	400 torr at 20.6 °C	Budavari et al. 1996
Vapor density (air=1)	2.0	Hartung 1994
Melting/boiling point	−45 °C/39.1 °C	EPA 1986
Solubility in water	0.067 g/mL	Hartung 1994
Conversion factors in air	1 ppm=2.34 mg/m^3 1 mg/m^3=0.43 ppm	NIOSH 1997
Reactivity	Exothermic reaction with water can lead to explosion	EPA 1986

Numerous animal studies corroborate the epidemiological findings in humans. Unlike other isocyanates, MIC is not a sensitizer.

Selected physicochemical properties of MIC are listed in Table 3–2.

2. HUMAN TOXICITY DATA

2.1. Bhopal Disaster

On the night of December 2/3, 1984, an estimated 30 tons of MIC gas were released over Bhopal, India from a carbamate factory when water entered the storage tank. The reaction of water and MIC is exothermic and resulted in increased pressure and temperature until the tank's safety valve ruptured. Total duration of the release was approximately 1 h. Atmospheric conditions maintained the MIC cloud close to the ground, and light winds moved it towards a heavily populated area. Dispersion calculations by Karlsson et al. (1985) estimate concentrations of MIC to have ranged from

3,000 ppm at 270 m downwind to 10 ppm at 5,500 m downwind. However, the concentrations to which the population was actually exposed are unknown.

The official death toll was 2,250 individuals, with another 50,000 incapacitated and about 100,000 treated in area hospitals. In addition, about 1,000 livestock were killed. The area with the heaviest casualties was 6–7 km^2 south of the factory and severe injuries occurred in a region of about 25 km^2 (Karlsson et al. 1985). In general, deaths were not instantaneous but occurred in phases over the next few days following the release. Only a few deaths were recorded within the first few hours; a second phase occurred between 8 and 12 h, and the greatest number of deaths occurred between 24 and 72 h after the MIC release (Varma 1989; Varma and Guest 1993).

Numerous accounts have been published detailing the effects of MIC on the population. The most frequently reported symptoms were burning and/or watering of the eyes, coughing, respiratory distress, pulmonary congestion, nausea, vomiting, muscle weakness, and CNS involvement secondary to hypoxia (Kamat et al. 1985; Misra et al. 1987; Lorin and Kulling 1986; Andersson et al. 1988; Weill 1988; Kamat et al. 1992). The frequency of reports of cough as an initial symptom most closely followed the distribution of deaths in the exposed populations (Andersson et al. 1988), deaths resulting from pulmonary edema (Weill 1988) or cardiac arrest following pulmonary edema (Varma and Guest 1993). Between areas in which deaths occurred and areas where only symptoms were reported, distance to the factory was a contributing factor, but the duration of exposure (up to 4 h) did not appear to vary (Andersson et al. 1988). Although most survivors improved within 2 weeks (wk), many had restrictive respiratory function with radiographic changes suggestive of interstitial deposits (Kamat et al. 1985).

Long-term health effects from the accident have been reported for populations followed for up to 3 years (y). One hundred and five days after the accident, a survey of children residing within 2 km of the factory at the time of the accident showed that 83.5% had persistent cough, 47.5% had breathlessness, 48.1% had rhonchi, and 43.2% had wheezing compared with abnormal respiratory findings in 8.5% of children living 8–10 km away; abdominal pain and anorexia were also increased by 8.0–9.8% in children living closer to the factory (Irani and Mahashur 1986). Another survey, which included adults and children, documented persistent respiratory, ophthalmological, neuromuscular, and gastrointestinal symptoms 15

wk after the accident (Naik et al. 1986). In more heavily exposed groups (defined by distance to the factory or number of symptoms), cognitive functions were impaired and consolidations were observed on chest radiographs after 1 y (Misra and Kalita 1997), with breathlessness, chest pain, and nausea/vomiting more frequent after 3 y (Andersson et al. 1990). Small airway obstruction, as measured by reductions in pulmonary function tests and/or abnormal chest radiographs, were found in victims at 1 y (Misra and Kalita 1997), 2 y (Kamat et al. 1992), and 10 y (Cullinan et al. 1997) after the accident. A study carried out 1–7 y after the accident found reductions in pulmonary function correlated with increases in inflammatory cells measured in bronchoalveolar lavage fluid and with radiographic abnormalities (Vijayan and Sankaran 1996).

Immediate and long-term ocular toxicities were also a major consequence of the MIC release in Bhopal. However, no cases of blindness were attributed to exposure (Andersson et al. 1984, 1985, 1988). Immediately after the accident, tearing, photophobia, profuse lid edema, and superficial corneal ulceration were reported (Andersson et al.,1984; Dwivedi et al. 1985). Superficial interpalpebral erosion of the cornea and conjunctiva observed initially (Andersson et al. 1988) was followed about 2 mo later by the typical whorling pattern of new growth and healing of the corneal epithelium (Andersson et al. 1984, 1985). Results showed that the incidence of cataract, conjunctivitis, corneal opacity, and hyperemia of the conjunctivae remained increased 3 mo after the accident in individuals residing within 2 km of the factory. In this survey, males were more affected than females, which the author attributed to the fact that the males ran in an attempt to find safety, and thus, increased their exposure, leaving the females behind in whatever shelter was available (Maskati 1986). From a follow-up study 3 y after the accident, Andersson et al. (1990) concluded that “Bhopal eye syndrome” may include full resolution of the initial interpalpebral superficial erosion, a subsequent increased risk of ocular infections, hyperresponsive phenomena such as irritation, watering, and phlyctens, and possibly cataracts. Eye irritation, reduction in vision, and persistent corneal opacity were also reported after 6–9 mo (Raizada and Dwivedi 1987) and after 2 y (Khurrum and Ahmad 1987).

Of interest here are reports comparing the effects of MIC with effects following exposure to related chemicals. In general, isocyanate exposures produce immunologic sensitization. However, evaluation of sera from Bhopal patients revealed low antibody titers that were transient (Karol et al. 1987). In a comparison to hydrogen cyanide, the absence of cyano

methemoglobin was noted (Misra et al. 1987), as was the fact that MIC pulmonary lesions were not characteristic of those seen after cyanide intoxication (Varma 1989; Weill 1988).

Based on model predictions, the actual consequences in Bhopal, and limited animal and human toxicity data, Karlsson et al. (1985) estimated the effects of MIC in humans from "short exposure" durations (Table 3–3). Although the authors did not define "short" duration, their models were based on 1 h, which was the approximate duration of the release. It is interesting to note that the Karlsson et al. (1985) results, especially for irritation of mucus membranes, are in close agreement with the results of controlled human inhalation studies described below.

2.2. Nonlethal Toxicity

2.2.1. Case Reports

Case reports have been submitted to EPA under TSCA sections 8D and 8E. One letter included a compilation of case reports in which industrial workers consistently noted skin and respiratory irritation from MIC exposure. There were no definitive cases of dermal or respiratory sensitization. All MIC-exposed workers apparently recovered completely when removed from the source of exposure. No exposure concentrations or durations were given (Union Carbide 1973). Another letter (Union Carbide 1966) described possible sensitization in a worker analyzing MIC by infrared spectroscopy. This individual had severe swelling and redness of the face after handling the chemical, but was not aware of exposure at the time (i.e., no odor or irritation was reported). Details of concurrent exposures and/or confounding factors were not included.

2.2.2. Experimental Studies

The odor threshold for MIC in air is 2.1 ppm (EPA 1986; AIHA 1989).

Four subjects were each exposed for 1–5 min to MIC at 0.4, 2, 4, or 21 ppm (Kimmerle and Eben 1964). At 0.4 ppm, no odor was detected and no irritation was reported by any of the volunteers. Minor but distinct irritation of mucous membranes (particularly lacrimation) was noted without odor at 2 ppm. Ocular irritation became more pronounced at 4 ppm. Exposure to 21 ppm was intolerable for even a moment.

TABLE 3–3 Symptoms of MIC at Short Exposure Times[a]

Concentration (ppm)	Effect in Humans
10	Irritation
30	Risk for severe injuries
100	Severe injuries and increased risk of death
300	Fatal

[a]Exposure times ≤1 h.
Source: Reproduced from Karlsson et al. 1985.

Eight volunteers were exposed to MIC at an analyzed concentration of 1.75 ppm for 1 min (Mellon Institute 1970). All individuals reported eye irritation, seven had tearing, and three reported nose and throat irritation. Complaints of these effects ceased within 10 min, except that one woman reported a sensation of "something in her eye" for 45 min. Six of the same individuals were subsequently exposed to MIC at 0.5 ppm for 10 min. All reported eye irritation, five had tearing, four had nose irritation, and two reported throat irritation. One person detected an odor after 3 min. Additional experimental details were not available.

In a slightly larger study, seven male volunteers were exposed to nominal concentrations at 0.3, 1.0, 2.5, or 5.0 ppm for 1 min or 1 ppm for 10 min (Mellon Institute 1963a). No effects were reported for 0.3 or 1.0 ppm for 1 min. Exposure at 2.5 and 5.0 ppm resulted in eye irritation in 4/7 and 7/7, nose irritation in 2/7 and 2/7, and tearing in 1/7 and 7/7, respectively. Throat irritation was also reported by one individual, and 3/7 could detect an odor during exposure at 5.0 ppm. During the 10-min exposure at 1 ppm, eye irritation and tears were reported in 7/7 individuals by 4 and 5 min, respectively, and nose and throat irritation were reported by 3/7 after 9 min of exposure; no odor was detected.

2.3. Developmental and Reproductive Toxicity

A door-to-door survey was carried out 4.5 mo after the accident in Bhopal, India, to determine the reproductive outcomes of women who were pregnant at the time of the release; the data obtained consisted of self-reported symptoms and pregnancy outcomes. The spontaneous abortion rate was 24.2% in the exposed area versus 5.6% in a control area. No specific

pattern of congenital defects was observed in term infants (reviewed in Arbuckle and Sever [1998] and Shepard [1995]). Similar results were obtained in a survey conducted approximately 9 mo after the accident (Varma 1987). Among 865 women reporting that they were pregnant at the time of the accident, 43.8% of these pregnancies did not result in a live birth; background rates of spontaneous abortion were not given. Of the 486 live births, 14.2% of the infants died within 30 d as compared with a background infant death rate of 2.6–3%. None of these surveys reported stage of pregnancy at the time of exposure, severity of maternal symptoms, or concentrations or duration of exposure.

2.4. Genotoxicity

At 1,114 d after the MIC release in Bhopal, cytogenetic studies were conducted on peripheral blood lymphocytes from exposed men and women (Ghosh et al. 1990). The frequency of chromosomal aberrations was generally greater in exposed individuals, with females showing a higher incidence than males. Nondisjunction was rare and frequencies of sister chromatid exchanges (SCE) and depression in mitotic and replicative indices could not be related to exposure.

In another study examining blood lymphocytes from MIC-exposed individuals, SCE frequencies were increased more than 3 times compared with a control population, and chromosomal breaks were observed in 10 of 14 (71.4%) MIC-exposed individuals versus 6 of 28 (21.4%) unexposed controls (Goswami 1986).

2.5. Carcinogenicity

No information was found regarding the carcinogenic potential of MIC in humans.

2.6. Summary

Although 2,250 deaths were reported following the MIC release in Bhopal, the concentration of MIC causing lethality in humans is unknown. Dispersion calculations estimated airborne concentrations to range from

3,000 ppm at 270 m downwind to 10 ppm at 5,500 m downwind. Persistent ocular and pulmonary pathology has been described for the survivors of the accident. There was an increase in self-reported spontaneous abortions and a decrease in the number of live births among women pregnant at the time. The chemical has been confirmed to be an irritant to mucous membranes in both experimental and epidemiological studies. Eye irritation, usually with lacrimation, was the most common symptom reported in controlled inhalation studies at concentrations of MIC ranging from approximately 1 ppm to 5 ppm. MIC has not definitively been shown to be a sensitizer.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

LC_{50} values for guinea pigs, rats, and mice are summarized in Table 3– 4. An LC_{50} for rabbits was not found in the available literature. Acute lethality studies, including clinical effects where available, are discussed below by species.

3.1.1. Rabbits

Two rabbits per group were exposed to MIC at 5.4 ppm for 6.75, 3.5, or 2 h or at 1.8 ppm for 7 h (Dow Chemical 1990). Although the concentrations were listed as nominal, the report stated that the analytical method (infrared absorption) was adequate for monitoring concentrations as low as 1.8 ppm. A description of the exposure chamber was not included. All animals exposed at 5.4 ppm died 1–2 wk postexposure, apparently due to respiratory infections. Animals exposed at 1.8 ppm survived. At 5.4 ppm for durations ≥3.5 h, the eyes were red and had evidence of corneal injury when observed with fluorescein. Ocular damage was slight in animals exposed at 5.4 ppm for 2 h and equivocal in animals exposed at 1.8 ppm for 7 h. No experimental details or further discussion was included.

Male albino rabbits were exposed in a flow-through chamber to a monitored concentration of MIC at 1,260 ppm for 30 min (Pant et al. 1987). Although numbers of deaths were not stated, it is unlikely most animals survived exposure at this concentration. At necropsy, lung weights were

TABLE 3–4 Summary of LC50 Values

Species (Gender)	Number of Animals Per Group	LC_{50} (ppm)	Duration (h)	Reference
Guinea pigs (male and female)	6/gender	5.4	6	Dodd et al. 1985, 1986
Guinea pigs (not stated)	Not stated	10.6	4	Mellon Institute 1970
Guinea pigs (male)	8	26.5	3	Ferguson and Alarie 1991
Rat (male and female)	6/gender	6.1	6	Dodd et al. 1985, 1986
Rat (not stated)	6	17.5	4	Mellon Institute 1970
Rat (male)	6	11.1	4	Fait and Dodd 1981
Rat (female)	6	11.0	4	Fait and Dodd 1981
Rat (not stated)	20	5	4	Kimmerle and Eben 1964
Rat (not stated)	6	27.4	2	Mellon Institute 1970
Rat (not stated)	20	21	2	Kimmerle and Eben 1964
Rat (not stated)	6	41.3	1	Mellon Institute 1970
Rat (male)	20	45.01	1	ManTech Environmental 1992
Rat (female)	4	171	0.25	Dodd et al. 1987
Mouse (male and female)	6	12.2	6	Dodd et al. 1985, 1986
Mouse (male)	8–41	26.8	3	Varma et al. 1988
Mouse (male)	6	112.4	0.5	Vijayaraghavan and Kaushik 1987

increased 2- to 2.5-fold, and the lungs had large hemorrhagic patches. Histologically, the epithelial lining of the bronchioles was necrotic and sloughed, and the alveoli were edematous.

3.1.2. Guinea Pigs

Groups of eight male English short-haired guinea pigs were exposed for 3 h to analytical concentrations of MIC at 6, 13, 19, 27, or 37 ppm (Ferguson and Alarie 1991). Each animal was held in a whole body plethysmograph attached to a primary chamber into which MIC was delivered. A 3-h LC_{50} of 26.5 ppm was calculated. Pulmonary performance, as measured by respiratory frequency, amplitude, coughing, flow-volume measurements, O_2 uptake, and CO_2 output, was evaluated for up to 1 y in the survivors. At 19 ppm and 37 ppm, 2/8 and 6/8 animals exposed died within 48 h, respectively; no animals survived more than 24 h following exposure at 27 ppm. Coughing was observed in animals exposed at ≥19 ppm; the frequency increased with concentration and persisted in survivors for more than 5 d postexposure. Deterioration of pulmonary performance (decreased breathing frequency and abnormal flow-volume loops) was observed in animals exposed at 6 ppm and 13 ppm, but complete recovery occurred within a few weeks. Because all measures of pulmonary performance in the 6- and 13-ppm animals returned to normal, these groups were terminated at 1 and 2 mo postexposure, respectively. Gross necropsy was unremarkable in the animals exposed at 6 ppm and 13 ppm. Histologically, the surface density of the epithelial layer of all conducting airways was decreased at 6 ppm after 1 mo but had returned to normal in the 13-ppm animals after 2 mo. In animals surviving exposure at 19 ppm or 37 ppm, impairment of pulmonary performance, indicative of chronic obstructive lung disease, was observed after 1 y. These higher exposure groups had concentration-related decreases in tidal volume and abnormal flow-volume loops in response to CO_2 challenge. At necropsy 1 y postexposure, the lungs from the animals exposed at 19 ppm or 37 ppm showed large areas of scarring, and portions of the lobes appeared atelectatic. Histologically, there was an increase in fibrous connective tissue in the main bronchi, an increase in septal thickness of the alveoli, and destruction of the alveolar walls, all of which were more severe at 37 ppm than at 19 ppm. In a companion study to the one just described, no evidence of inhibition of oxygen utilization (cyanide-like effect) was reported for the 37-ppm group (Alarie et al. 1987).

Dodd et al. (1985, 1986) determined a 6-h LC_{50} of 5.4 ppm for male and female guinea pigs (*N*=6). Analytically determined mean exposure concentrations in the flow-through chamber were 0, 1.0, 2.4, 5.4, and 10.5 ppm. No adverse effects were observed at 1 ppm and 2.4 ppm. Clinical signs included lacrimation and/or perinasal wetness at ≥5.4 ppm and irregular or labored breathing at 10.5 ppm. In animals that died, red nasal discharge was observed and the lungs were grossly hyperemic. Histologically, congestion, necrosis, and rhinitis of the nasal cavity, necrosis of the larynx and trachea, and congestion, hemorrhage, edema, hyaline membrane formation, and necrosis of the lungs were observed. The bronchioles were nearly obliterated by exfoliated epithelial cells. Severity of these lesions was concentration-related, and epithelial regeneration was observed in survivors (Fowler and Dodd 1985, 1986).

Female Hartley guinea pigs (minimum of four per group) inhaled 25, 125, 225, or 675 ppm for 15 min in a static exposure chamber (Dodd et al. 1987). Chamber concentrations were analytically determined prior to exposure. The 15-min LC_{50} was calculated as 112 ppm, and the approximate time to death was 1–3 d postexposure. Number of deaths at each concentration was not given. Clinical signs at concentrations ≥225 ppm included lacrimation, nasal wetness, rubbing of eyes and nose with forepaws, partial to complete closure of the eyelids, salivation, mouth breathing, and short periods of hyperactivity; prior to death animals were prostrate and gasping. Histologically, necrosis of the epithelia lining the conducting airways resulted in sloughing of large sheets of epithelial cells (Fowler et al. 1987). Sheets of epithelial cells, together with fibrin and mucus, occluded the more distal airways causing atelectasis. The severity of the lesions was concentration-related.

Male Hartley guinea pigs (group sizes not stated) were exposed to target concentrations of MIC at 25, 125, or 225 ppm for 15 min in a static exposure chamber, and blood samples were taken immediately or up to 16 h postexposure (Troup et al. 1987). All animals in the 225-ppm group died prior to the 16-h sample, and only one animal in the 125-ppm group survived; time to death was not stated. No effects were observed on red blood cell cholinesterase activity. Increased hemoglobin concentration and hematocrit developed in animals exposed at ≥125 ppm. At the highest concentration, neutrophilia and increases in creatine kinase were observed. These changes were indicative of a generalized hypoxic injury with concomitant pathophysiologic alterations.

In an immunotoxicity study, five pairs of female Sprague-Dawley guinea pigs were exposed in a static exposure chamber to a target concentration at 650 ppm (644–702 ppm) until one of the pair died (11–15 min) (Kolb et al. 1987). The survivor was sacrificed, and plasma was obtained from both animals and analyzed for complement consumption. In both animals, complement activation had occurred as indicated by a reduction in CH_{50}, C3, and C5 activity levels. Plasma protein concentrations were significantly elevated in animals that had died but not in animals that were sacrificed.

Groups of four male guinea pigs were exposed to "metered" (nominal) concentrations of MIC at 15.63, 31.25, or 62.5 ppm for 4 h followed by a 14-d observation period (Mellon Institute 1966). A description of the exposure chamber was not included. All animals died following exposure to the highest concentration, 3/4 died after exposure at 31.25 ppm, and 0/4 died after exposure at 15.63 ppm; deaths occurred within 48 h. Animals showed immediate signs of ocular and nasal irritation at the "higher" concentrations and were gasping after 10 min at 62.5 ppm. Gross necropsy revealed hemorrhage of the lungs. Further experimental details were not given. A 4-h LC_{50} of 10.6 ppm was reported for guinea pigs, but experimental details were not included (Mellon Institute 1970).

3.1.3. Rats

Groups of 20 male Fischer rats were exposed in whole-body, flow-through chambers for 1 h to analyzed concentrations of MIC at 22.7, 33.5, 46.5, 49.7, or 62.5 ppm (ManTech Environmental 1992). Mortalities were 0, 4, 7, 16, and 16 animals, respectively, resulting in a calculated 1-h LC_{50} of 45 ppm with 95% confidence limits of 38.62–52.46 ppm. Most rats in all exposure groups exhibited dyspnea, rales, salivation, lacrimation, and clear or red nasal discharge upon removal from the chamber. Clinical signs during the 14-d observation period included redness around the nose and eyes, gasping, wheezing, cold to touch, rough hair coat, diarrhea, discolored inguinal fur, and emaciation. All surviving rats lost weight during the first week postexposure, and survivors exposed at $\geq$33.5 ppm continued to have body-weight loss during the second week. Gross findings in rats that died included red or pale puffy lungs, gas-filled stomach, and yellow and gas-filled intestines. Puffy, pale or red lungs, tan or gray areas, and/or red foci in the lungs were observed in all animals necropsied at the end of the observation period.

Dodd et al. (1985, 1986) determined a 6-h LC_{50} of 6.1 ppm for male and female Fisher 344 rats combined (*N*=6/gender/group). Exposure was whole-body, and analytically determined exposure concentrations in the flow-through chamber were 1, 2.4, 5.4, 10.5, and 20.4 ppm. Clinical signs included lacrimation and/or perinasal wetness, irregular or labored breathing, decreased activity, and ataxia as well as body-weight loss during the observation period. Gross observations included dark red to brown material encrusted around the mouth, nares, and eyes and lung congestion. In animals that died, congestion, epithelial necrosis, and inflammation of the nasal tissues, larynx, trachea, and lungs with edema in the lungs were confirmed histologically. Squamous metaplasia in the upper airways and submucosal fibroplasia in the bronchi and bronchioles became more pronounced in animals with longer survival times (Fowler and Dodd 1985, 1986).

Four-hour LC_{50}s of 11.1 ppm and 11.0 ppm were calculated for male and female Fisher 344 rats (*N*=6/gender), respectively (Fait and Dodd 1981). Exposure was whole-body and analytically determined exposure concentrations in the flow-through chamber were 5.2, 15.24, 25.6, and 36.07 ppm. Clinical signs during and postexposure included respiratory difficulty, lacrimation, nasal discharge, dark red staining of the fur in the perioral and perinasal regions, and decreased motor activity; the severity of these signs was concentration-related. Gross necropsy revealed the lungs to be dark red with mottling.

Two- and four-hour LC_{50}s for rats (strain not specified) were calculated to be 21 ppm and 5 ppm, respectively (Kimmerle and Eben 1964). The 2-h value was based on analytical concentrations of MIC at 2, 20, and 21 ppm that resulted in deaths of 0/20, 10/20, and 10/20 animals, respectively. The 4-h value was based on analytical concentrations of 5, 9, and 23 ppm, which killed 10/20, 16/20, and 20/20 animals, respectively. All deaths occurred 1–7 d after exposure. Signs of toxicity at concentrations >2 ppm included mucous membrane irritation (not further described), labored breathing, and lung edema. In an additional experiment by the same authors, exposure at 22 ppm for 1 h resulted in the death of 7/20 in 3–8 d with similar clinical signs.

Four-hour exposures of Wistar rats to MIC at 62.5 ppm killed 6/6 animals within 2 d, but no deaths occurred following exposure at 31.2 ppm (Mellon Institute 1963b). A description of the exposure chamber was not included. This range-finding data was used as the basis for LC_{50} studies in which groups of six male Wistar rats were exposed to varying concentrations for durations of 7.5 min to 4 h (Mellon Institute 1970). At concentra

tions of ≥8.9 ppm (durations not specified) the animals exhibited signs of eye, nose, and lung irritation, including gasping and labored breathing. Transient eye and nose irritation was observed during exposure at 4.47 ppm. LC_{50} values for 4 h, 2 h, 1 h, 30 min, 15 min, and 7.5 min were listed as 17.5, 27.4, 41.3, 76.6, 216, and 541 ppm, respectively. Although additional experimental details were not given, the report stated that the results were based on analytically verified concentrations.

Female Wistar rats (*N*=5 to 8) were exposed whole-body to analytical concentrations of MIC at 297, 420, 528.7, or 665.7 ppm for 30 min in a static exposure chamber (Vijayaraghavan and Kaushik 1987). The 30-min LC_{50} was calculated as 439.5 ppm. The cyanide antidote sodium thiosulfate, given before or after exposure, had no effect on mortality. The histological changes in the lungs were reported in another study in which animals were exposed in static chambers to approximately 1, 0.5, and 0.33 times this 30-min LC_{50} (Pant et al. 1987). Rats that survived had granular deposition of the lining cells of the bronchioles and epithelial necrosis leading to desquamation of the bronchiolar epithelium and congestion and edema. The severity of the lesions increased with MIC concentration.

Female Sprague-Dawley rats (minimum of four per group) were exposed whole-body at 100, 600, or 1,000 ppm (analytically determined) for 15 min in a static exposure chamber (Dodd et al. 1987). The 15-min LC_{50} was calculated as 171 ppm and the time to death was 1–3 d postexposure. Clinical signs at concentrations ≥600 ppm included lacrimation, nasal wetness, rubbing of eyes and nose with forepaws, partial to complete closure of the eyelids, salivation, and mouth breathing. Histologically, necrosis of the epithelia lining the conducting airways resulted in sloughing of large sheets of epithelial cells (Fowler et al. 1987). The sheets of epithelial cells, together with fibrin and mucus, plugged the more distal airways causing atelectasis. The severity of these lesions was concentration-related.

Female rats (strain not specified; *N*=4) were exposed to concentrations of MIC ranging from 1.8 to 230 ppm for durations of 0.1–7 h (Dow Chemical 1990). Although the concentrations were listed as nominal, the report stated that the analytical method (infrared absorption) was adequate for monitoring concentrations as low as 1.8 ppm; however, the analytical concentrations were not reported. A description of the exposure chamber was not included. Exposures at 230 ppm for ≥0.5 h, 90 ppm for ≥2 h, 35 ppm for ≥4 h, and 13.8 ppm for ≥4 h resulted in the deaths of all animals. Exposures resulting in some or no mortalities are as follows: 230 ppm for 0.2 h, 1/4; 230 ppm for 0.1 h, 0/4; 90 ppm for 1 h, 3/4; 35 ppm for 2 h, 3/4;

35 ppm for 1 h, 0/4; 13.8 ppm for ≤2 h, 0/4; 5.4 ppm for 7 h, 3/4; 5.4 ppm for ≤4 h, 0/4; 1.8 ppm for ≤7 h, 0/4. At concentrations ≥5.4 ppm, clinical signs indicated eye and nasal irritation, and necropsy revealed moderate to slight lung congestion. Liver and kidney pathology was also stated, but not defined, for most of these animals. No irritation was observed from exposures at 1.8 ppm for 4 or 7 h with questionable to slight lung pathology observed at necropsy.

Male and female F344/N rats (*N*=5/gender/time point) were exposed to MIC at 0, 3, 10, 15, 20 or 30 ppm for 2 h and necropsied at various time points up to 91 d postexposure (Bucher et al. 1987a). Exposure was whole-body in flow-through chambers. Chamber concentrations were monitored continuously and did not vary more than ±10% of nominal. Clinical signs during exposure included irritation and restlessness with rubbing of the eyes and ears at concentrations of 10–15 ppm; at 20 ppm, rats walked with a low carriage and the eyes were partially closed; at 30 ppm, animals lay flat with their eyes closed, had excessive lacrimation, and a frothy, reddish discharge was seen from the nose and mouth. Upon removal from the chamber, concentration-related clinical signs included weakness, ruffled fur, respiratory distress (gasping, moist rales, open mouth, abdominal breathing), and decreased body-weight gain. No animals died during exposures, but deaths were observed 15–18 h later in the 20- and 30-ppm groups, with a higher proportion of males dying during the first 3 d. These initial deaths were followed by a 5- to 7-d period in which few deaths occurred before more animals began dying. All male rats died by day 28 and only two female rats survived to 91 days following exposure at 30 ppm. Survival at 10 ppm was approximately 70% for both males and females, and at 20 ppm it was approximately 25% and 40% for males and females, respectively. Because animal survival was presented in graphic form, the exact numbers could not be discerned and control data were not included. At necropsy, lung weights were increased in the 10- and 30-ppm males and in the 30-ppm females throughout the postexposure period. Additional investigations showed no clinical chemistry or hematologic changes, no inhibition of blood or brain cholinesterase, and no effect on lethality following sodium nitrite and sodium thiosulfate administration (Bucher et al. 1987a). No gross or microscopic evidence of epithelial erosion or ulceration of the cornea or adjacent tissues was observed at any time following exposure (Gupta et al. 1987).

Tissues from the rats in the Bucher et al. (1987a) study discussed above were examined for gross and histopathological changes immediately after

exposure at 10 ppm or 30 ppm and through 91 d; a 2-h, 3-ppm group was also included (Bucher et al. 1987b). The severity of all lesions was concentration- and gender-related, and the 30-ppm males were the most affected. Grossly, reddish white encrustations were observed around the mouth, nose, and eyes of dead and moribund animals. The middle and median lobes of the lung were commonly consolidated, and petechiae and ecchymoses were observed in the lungs of the 30-ppm group. Microscopic lesions in animals exposed at 3 ppm included necrosis of the respiratory epithelium of the trachea, but the lesions were essentially resolved by day 14. At concentrations of 10 ppm and 30 ppm, necrosis of both respiratory and olfactory epithelium was followed by inflammation, epithelial regeneration, and intraluminal fibrosis. The severity of the inflammatory lesions decreased over time, but intraluminal fibrosis, mild bronchitis and bronchiolitis, and mucous plugs persisted throughout the 91-d period (Bucher et al. 1987b). Necrosis and degeneration followed by regeneration of both the respiratory and olfactory epithelia of the nasal mucosa were confirmed by ultrastructural analysis with transmission electron microscopy (Uraih et al. 1987).

Groups of six male albino rats were exposed whole-body to nominal MIC concentrations at 8, 16, 32, or 64 ppm for 6 h followed by a 15-d observation period (IRDC 1964). In addition, two animals in the 16-ppm group were sacrificed at 24 h for interim histopathological evaluation. Exposure concentrations were calculated on the basis of air flow through the chamber and the amount of material vaporized. Slight nasal irritation was noted in animals exposed at 8 ppm, and all animals survived. At ≥16 ppm, animals exhibited labored breathing and signs of respiratory distress. All animals died within 24 h of exposure to 64 ppm, and by the end of the observation period, 5/6 and 1/4 animals in the 32- and 16-ppm groups, respectively, died. Hemorrhagic areas were observed at necropsy in the lungs of all rats exposed at ≥16 ppm. After the 15-d observation period, histopathological evaluation revealed inflammatory lesions in the lungs of all exposed rats, the severity of which was concentration-related. Rats that died or were sacrificed during the first or second postexposure day had pulmonary edema, early acute bronchitis, congestion, and hemorrhage.

Male LAC:P rats (N=3/concentration/time point) were exposed whole-body to MIC for 1 h in a static exposure chamber, and lesions of the respiratory tract were assessed at various time points over 3 wk. Nominal chamber concentrations were 8.4, 42, 105, 210, or 420 ppm (Nemery et al.

1985a; Dinsdale et al. 1987). During exposure, all animals showed signs of eye and nose irritation and had a clear nasal exudate. A concentration-related decrease in respiratory rate led to respiratory acidosis and hypoxemia. Within 24 h, one rat exposed at 210 ppm died and three exposed at 420 ppm died; four others were killed moribund. Following exposure at 105 ppm, one animal died 10 d postexposure, and another died 6 wk postexposure. Animals exposed at 105 ppm were examined for ultrastructural changes in the respiratory tract. The authors stated that a "raft" of cellular debris and fibrin lined most of the airways during the first week after exposure, but repair to the underlying epithelium was well advanced within 2–3 d. The majority of airways were lined by a normal epithelium within 3 wk postexposure, but isolated foci of hyperplasia were present, and a few of the bronchioles were blocked by fibrin, although others showed signs of residual peribronchial fibrosis. The study authors stated that the initial chamber concentrations "must be reduced by a factor of about four" to obtain the time-weighted average (TWA) concentrations over 1 h. Their explanation for this statement was that an initial injection of MIC into the tank gave the reported nominal concentration, but that did not reflect the real TWA concentration over the 1-h exposure. No data were included to support this statement.

Male Lister hooded rats were exposed, in pairs, for 2 h to MIC at 11, 21, 31, or 65 ppm (Salmon et al. 1985). Exposures were whole-body in flow-through chambers. No animals died during exposure, but at 65 ppm, one rat died 45 h after exposure and the other was killed *in extremis* at 50 h. Necropsy of these animals revealed hemorrhagic patches on the lungs and pulmonary edema. During exposures animals exhibited slow and irregular breathing and were inactive; the severity of the clinical signs was concentration-related. When examined 20 h postexposure, erosions of the corneal epithelium were observed in all animals exposed at ≥21 ppm. The surviving animals were then followed for up to 14 mo (Gassert et al. 1986). One rat exposed at 31 ppm died at 6 mo, and one exposed at 11 ppm died at 8 mo, following sudden onset of respiratory distress. Necropsy of exposed animals at 14 mo showed a history of mild respiratory infection in all animals as evidenced by lymphoid hyperplasia adjacent to bronchiolar airways. Mild interstitial fibrosis in the peribronchiolar regions was seen in all animals. In the eyes, eosinophil and lymphoid infiltrate was most prominent in the animals exposed at 21 ppm.

Pairs of Charles Foster rats were exposed to MIC at 3.52 or 35.32 ppm for 10 min and necropsied immediately after death (Sethi et al. 1989).

During exposure, dyspnea, congestion in the eyes, bloody lacrimation, and nasal secretion were observed. Animals exposed at 35.32 ppm died within 4 min of exposure, and animals exposed at 3.52 ppm died 39 min and 293 min postexposure. Lung hemorrhages, dilated and congested trachea, pulmonary edema, and brain edema were observed at necropsy. The main histological findings were necrosis of the bronchial epithelium, lung edema, and congestion of the vessels in several visceral organs. It should be noted that for these exposure concentrations, the effects are more severe than (and are not consistent with) those reported in other similar studies. Additional details on the exposure system, generation of the test atmospheres, and monitoring of chamber concentrations were not provided.

Histological lesions in the lungs of male Lister rats were assessed up to 10 wk following a single 30-min exposure to nominal concentrations at 233, 465, or 930 ppm in static exposure chambers. The concentration of 465 ppm was stated as the LC_{50}, but details concerning the LC_{50} experiment were not reported (Jeevaratnam and Sriramachari 1994; Sriramachari and Jeevaratnam 1994). During exposures, the animals displayed acute respiratory distress. At 24-h postexposure, the numbers of deaths were 0/8, 4/10, and 7/12, respectively. Initially, acute necrotizing bronchitis of the respiratory tract accompanied by congestion, hyperemia, and interstitial and alveolar edema were observed with the severity related to concentration. This was followed at 4 wk by regeneration of the bronchial epithelium, but persistent interstitial pneumonitis, thickened alveolar septa, and areas of atelectasis were prevalent. At 10 wk postexposure, the only surviving animals were from the 233-ppm group. Those animals developed diffuse interstitial pulmonary fibrosis, but restoration of the bronchial epithelium was nearly complete. In a related study (Jeevaratnam et al. 1990), biochemical changes in rats were monitored up to 24 h after exposure at 465 or 930 ppm for 30 min. None of the animals in the high concentration group survived beyond 4 h postexposure. At both exposure concentrations, rats were afflicted with hyperglycemia, lactic acidosis, elevated plasma urea, and slight inhibition of plasma cholinesterase activity; erythrocyte cholinesterase activity remained unaffected.

Male rats (six per group) were exposed under a whole-body protocol at 1,344, 1,844, 4,607, 9,219, or 18,438 ppm (analytical concentrations) for 8 min in static exposure chambers. A series of toxicological studies was conducted on the surviving low-concentration animals. All animals died within 24 h after exposures at ≥4607 ppm. Mortality at 1,344 and 1,844 ppm was approximately 10–20% and 30–40%, respectively. Pronounced

clinical signs were indicative of severe irritation, and gross necropsy showed consolidation of the small lobes of the lung and distention of the gastrointestinal tract with gas. Food and water consumption and body weights of the survivors were initially reduced after exposure, followed by a gradual recovery. Peripheral emphysema and congestion of the alveolar capillaries persisted through 14 d postexposure with some reduction in severity over time (Dutta et al. 1988). Biochemical analyses conducted 7 or 14 d after exposure at 1,344 ppm showed an increase in total serum lactate dehydrogenase (Gupta et al. 1988), increases in lung levels of aniline hydroxylase and glutathione-S-transferase activities, decreased lung glutathione levels, increases in cytochrome P-450 and cytochrome b5 content in the lung (Mishra et al. 1988), impaired alveolar and peritoneal macrophage functions and delayed type hypersensitivity reactions (Dwivedi et al. 1988; Saxena et al. 1988), and an increased susceptibility to bacterial *E. coli* endotoxin (Saxena et al. 1988). In a companion study, alterations in the biochemical and cytological constituents of bronchoalveolar lavage fluid (BALF) were monitored over a period of 30 d following exposure at 1,344 ppm for 8 min (Gupta et al. 1991). Total protein, sialic acid, and lactic acid contents of BALF were increased followed by a gradual return to baseline between day 3 and day 30. Lactic dehydrogenase levels and the numbers of polymorphonuclear neutrophils increased over time.

Groups of three rats were exposed under a whole body protocol to high concentrations of MIC until death. The exposure concentration and timeto-death for all animals in each group were 171,600 ppm for 7 min (flow-through), 6,619 ppm for 18 min (flow-through), 8,580 ppm for 30 min (static), 832 ppm for 110 min (static), and 343 ppm for 196 min (static). Static exposure at 51 ppm for 6 h resulted in deaths of two of three rats by 8 d postexposure. Clinical signs included lacrimation, dyspnea, nasal discharge, and gasping (Eastman Kodak 1966).

3.1.4. Mice

Male Swiss-Webster mice were exposed using a whole-body regimen to MIC at 0, 9, 18, or 40 ppm for 3 h and were observed for 14 d following exposure (Varma et al. 1988). Concentrations in the flow-through chamber were monitored throughout exposure. Deaths in the MIC-exposed groups were 0/8, 5/11, and 31/41, respectively, for a calculated 3-h LC_{50} of 26.8 ppm. Clinical signs of toxicity were similar for all exposure groups with

the animals scratching their noses and lacrimating. The animals ceased activity and remained listless for the duration of exposure but could be aroused by tapping on the chamber. Decreased respiratory rates were observed but not quantified. Concentration-related reductions in body weights were observed 1–2 d after exposure followed by gradual recovery. However, in the 40-ppm group, recovery of body weights was followed by a second phase of decrease from day 7 to day 8 postexposure. In separate studies, mortalities at 40 ppm were delayed in mice that had been deprived of food for 24 or 48 h and were reduced in mice injected with 2 mg dexamethasone per kilogram body weight prior to MIC exposure. Death was not prevented by administrations of sodium thiosulfate, ethanol, and atropine before or dexamethasone after MIC exposure.

A 6-h LC_{50} of 12.2 ppm for male and female $B6C3F_1$ mice combined (N=6/gender) was reported by Dodd et al. (1985, 1986). Analytically determined exposure concentrations in the flow-through chamber were 1, 2.4, 5.4,10.5, and 20.4 ppm. Clinical signs at ≥5.4 ppm included lacrimation and/or perinasal wetness, irregular or labored breathing, decreased activity, ataxia, and/or hypothermia as well as body-weight loss in all treated groups during the observation period. Gross observations included mild perinasal exudate and discoloration of the lungs. In animals that died, congestion, necrosis, and inflammation of the nasal tissues, necrosis of the larynx and trachea, and edema and bronchiolar epithelial necrosis in the lungs were observed histologically. Squamous metaplasia in the upper airways and submucosal fibroplasia in the bronchi and bronchioles became more pronounced in animals with longer survival times (Fowler and Dodd 1985, 1986).

Male Swiss albino mice (N=6) inhaled analytical concentrations of MIC at 83.8, 94.0, 132.8, or 236.2 ppm for 30 min in a static exposure chamber (Vijayaraghavan and Kaushik 1987). The 30-min LC_{50} was calculated as 112.4 ppm. Sodium thiosulfate, given before or after exposure, failed to influence mortality.

Male and female $B6C3F_1$ mice inhaled MIC at 0, 3, 10, or 30 ppm for 2 h (Boorman et al. 1987a,b; Bucher et al. 1987a). Exposure was conducted using whole-body in flow-through chambers. Chamber concentrations were monitored continuously and did not vary more than ±10% of nominal. Five animals per group were necropsied within 3 h after exposure and on days 1, 3, 7, 14, 49, and 91. During exposures, animals were inactive, especially at 30 ppm, and dyspnea was observed upon removal from the chamber. Deaths began 15–18 h after exposure to MIC at 30 ppm fol

lowed by 5–7 d during which few deaths occurred. Sixteen of 80 (20%) males in the 30-ppm group died, with seven deaths occurring within the first 24 h. Two females died, one each in the 30- and 10-ppm groups. Lung weights of males exposed at 30 ppm were significantly increased beginning on day 3 postexposure (Bucher et al. 1987a). Treatment-related lesions of the respiratory system were observed in both genders, with the severity greater in males. At 30 ppm there was extensive erosion and necrosis of the epithelia in the nasal cavity, trachea, and main bronchi. In the nasal cavity, recovery was essentially complete with the exception of small areas in the olfactory epithelia where lesions persisted in the males through day 91. In the trachea and major bronchi, fibrin and cellular debris were present in the airways, which in some cases had organized and formed fibrotic projections. Fibrosis was more severe in the males and chronic alveolitis and atelectasis were evident. Similar but less severe lesions were seen at 10 ppm. Acute inflammation and erosion of the respiratory epithelium were the main effect at 3 ppm. Complete recovery occurred in mice exposed at 3 or 10 ppm (Boorman et al. 1987a,b). Degeneration followed by rapid regeneration of the respiratory and olfactory epithelia was also confirmed ultrastructurally by transmission electron microscopy for males exposed at 10 or 30 ppm (Uraih et al. 1987). In contrast to many of the victims of the accident in Bhopal, ocular lesions in mice were not found (Boorman et al. 1987a). Clinical pathology and hematology end points and blood and brain cholinesterase activities were not affected by treatment. The cyanide antidotes sodium nitrite and sodium thiosulfate were not effective against mortality (Bucher et al. 1987a).

3.2. Nonlethal Toxicity

3.2.1. Guinea Pigs

Tissue hypoxia and metabolic acidosis were observed in female Hartley guinea pigs (N=2 to 3) statically exposed at 240 or 628 ppm for 15 min (Fedde et al. 1987). Following exposure, the animals were anesthetized and artificially ventilated for measurement of blood gases. Both concentrations of MIC resulted in marked reductions in the partial pressure of oxygen and in arterial pH. The partial pressure of oxygen remained low after ventilation with 100% oxygen, indicating severe intrapulmonary blood shunting. Hemoglobin and hematocrit were not affected by this protocol. Gross ob

servation of the lungs showed atelectasis, multifocal hemorrhages, and long strands of viscous material in the trachea and primary bronchi.

Hypoxemia and metabolic acidosis were confirmed in another study in which the oxygen binding properties of the blood were determined following whole-body exposure of female Hartley guinea pigs (number not reported) to a mean concentration at 698 ppm (range 618–804 ppm) for 15 min in a static chamber (Maginniss et al. 1987). Erythrocyte volume, methemoglobin concentration, oxygen binding capacity, and combined red cell organic phosphate concentration were not affected. However, lactic acidosis was indicated by increased circulating lactate concentrations, and oxygen equilibrium curves were significantly right-shifted (reduction in hemoglobin-oxygen affinity). In consequence, hemoglobin-oxygen saturation decreased from 66% for the controls to 42% for the MIC-exposed animals resulting in hypoxia.

The potential of inhaled MIC to cause respiratory sensitization was investigated in guinea pigs (Mellon Institute 1970). Adult male albino guinea pigs were exposed to an analytical concentration at 1 ppm for 2 h/d, 3 times per week for 3 wk. A description of the exposure chamber was not provided in the Mellon report. After a 3 wk incubation interval, groups of seven animals were exposed for 2 h at either 1 or 5 ppm. No signs suggesting tracheal edema or other evidence of respiratory allergic response were observed.

3.2.2. Rats

Lesions in the respiratory tract of male F344 rats (*N*=5) were examined 7 d after a single 6-h exposure to a monitored concentration of MIC at 3 ppm (Mitsumori et al. 1987). Exposures were carried out using a whole-body protocol and flow-through chambers. A marked reduction in body weight was noted during the recovery period. Inflammation was seen throughout the respiratory tract with regeneration of the respiratory epithelium in the nasal passages and trachea. Erosion of the respiratory epithelium in the bronchi was present. Severe inflammation of the alveoli correlated with atelectasis.

Groups of 50 male and 50 female F344 rats were given a single 2-h exposure at 0, 1, 3, or 10 ppm and then held for 2 y (Bucher and Uraih 1989). Survival and body-weight gains of exposed animals were similar to the controls throughout the study. At the end of the 2-y period, 42% of

males and 36% of females exposed at 10 ppm had evidence of intraluminal fibrosis of secondary bronchi. It was not stated whether these were analytical or nominal concentrations of MIC; however, in other work by these authors, concentrations were reported as analytical. Also, a description of the exposure chamber was not given.

Pulmonary function (Stevens et al. 1987) was assessed through 13 wk and cardiopulmonary function (Tepper et al. 1987) was assessed at 4 and 6 mo after a single exposure of male F344 rats at 3, 10, or 30 ppm for 2 h (number not reported). Exposure was whole-body in flow-through chambers. Chamber concentrations were monitored continuously and did not vary more than ±10% of nominal. None of the animals exposed at 30 ppm survived beyond 1 wk, but details of deaths were not reported. Body weights of rats exposed at 10 ppm were significantly decreased throughout the 6-mo study. Several measures of lung volume were increased by exposure at 10 ppm. Total lung capacity was increased to 120% of control at 4 wk and to 140% of control by 13 wk postexposure. Residual volume and end expiratory volume were markedly increased through 13 wk. Single-breath diffusion capacity to carbon monoxide was depressed at 1 and 2 wk postexposure and distensibility of the lung was depressed at 1 wk, but these end points were similar to controls thereafter (Stevens et al. 1987). Minute ventilation was significantly increased during CO_2 challenge 4 mo after exposure but was similar to controls after 6 mo. Increased lung recoil was indicated by an increase in maximum expiratory flow and a decrease in expiratory time. Also at 4 mo postexposure, an increase in cardiac arrhythmias was observed in both the 3- and 10-ppm groups. At 6 mo, forced expiratory flow-volume curves indicated persistent airway obstruction. Wet and dry lung weights of rats exposed at 10 ppm were increased through 4 mo but were not reported for the 6-mo time point (Tepper et al. 1987).

Male Sprague-Dawley rats (number not reported) were exposed in a static exposure chamber to MIC at 100, 600, or 1,000 ppm for 15 min and blood samples were taken immediately or up to 16 h postexposure (Troup et al. 1987). Some animals in the 1,000-ppm group died prior to the 16-h sample, but the number and time to death were not stated. No effects were observed on red blood cell cholinesterase activity. Increases in hemoglobin concentration, hematocrit, erythrocytes, reticulocytes, mean corpuscular volume, neutrophils, and creatinine kinase occurred in animals exposed at 1,000 ppm. An increase in reticulocytes also occurred at 600 ppm. These changes were indicative of a generalized hypoxic injury with concomitant pathophysiologic alterations.

3.2.3. Mice

Sensory irritation to MIC was evaluated in male Swiss-Webster mice (*N*=4) exposed to analytical concentrations between 0.5 and 7.6 ppm for 90 min. Exposures were head-only in flow-through chambers. The RD_{50} (concentration that causes a 50% reduction in breathing rate) was 1.3 ppm (Ferguson et al. 1986). In the same study, pulmonary irritation was evaluated in tracheally cannulated (TC) mice. The RD_{50TC} was found to be 1.9 ppm (Ferguson et al. 1986). Another study using male ICR mice reported an RD_{50} of 2.9 ppm (James et al. 1987).

Groups of 50 male and 50 female $B6C3F_1$ mice were given a single 2-h exposure to MIC at 0, 1, 3, or 10 ppm and then held for 2 y (Bucher and Uraih 1989). Survival and body-weight gains of exposed animals were similar to the concurrent controls throughout the study. Permanent or chronic lung lesions were not described for mice. The publication failed to state whether the results were expressed as analytical or nominal concentrations of MIC; however, in other work by these authors (Bucher et al. 1987a), concentrations were reported as analytical. Also, a description of the exposure chamber was not given.

Female $B6C3F_1$ mice (*N*=10) were exposed whole-body in flow-through chambers to analytical concentrations of MIC at 0, 1, or 3 ppm for 6 h/d for 4 consecutive days, and systemic immunity was evaluated within 3–5 d (Luster et al. 1986). Humoral immunity (antibody response to sheep erythrocytes) and natural killer cell activity were not affected, and resistance to infectious agents (*Listeria monocytogenes,* mouse malaria parasite, influenza virus) or to B16F10 transplantable tumor cells was not compromised by exposure.

3.3. Developmental and Reproductive Toxicity

Pregnant Sprague-Dawley rats (*N*=11) were exposed whole-body in flow-through chambers to MIC at 9 ppm (analytical) for 3 h on gestation day (GD) 10 (Varma et al. 1990). Dams were sacrificed on GD 20 and the uterine contents examined for live fetuses and postimplantation loss. Group mean values for maternal body weights, fetal weights, and placental weights were significantly ($p \leq 0.05$; Student's t-test) reduced to 87%, 82%, and 85%, respectively, of the control levels. The total number of resorptions was significantly ($p \leq 0.05$) increased in the treated dams (76 versus 2 in the control group) resulting in a significant ($p \leq 0.05$) decrease

in the number of live fetuses; a total of 86 live fetuses were reported for the treated group versus 119 for the control group. Four of 11 (36%) treated dams had no live fetuses. Data for numbers of resorptions and live fetuses were only given as group totals and not on a per-litter basis. External, visceral, and skeletal examinations of the fetuses were not conducted.

Male and female Charles Foster rats (N=5) were exposed "once before mating" to MIC at 0, 0.212, 0.265, or 0.353 ppm for 30 min (Singh et al. 1994). Concentration-related decreases in maternal body weights occurred during gestation, and average fetal body weights and lengths were reduced in all treated groups. The percent resorptions was 3, 12, 20, and 32, respectively, based on group totals for numbers of implantations and resorptions; however it was not possible to discern from the data the number of resorptions per dam or the number of dams with whole litter resorption. The percent of fetuses (litter incidence not given) with limb defects and skeletal and visceral malformations was increased in the treated groups in a concentration-related manner. No information was given describing the exposure chambers, statistical procedures, test atmosphere generation, or concentration determinations. Therefore, the Singh et al. report was not utilized in weight-of-evidence considerations on MIC.

Pregnant Swiss-Webster mice (N=12 to 24) were exposed in flow-through chambers to analytically monitored concentrations of MIC at 0, 2, 6, 9, or 15 ppm for 3 h on GD 8 (Varma, 1987). The number of animals in each group was 24, 11, 12, 12, and 18, respectively. Dams were sacrificed on GD 18, the uterine contents were examined for postimplantation losses, and the live fetuses were examined for external, visceral, and skeletal abnormalities. Two dams in each of the 9- and 15-ppm groups died. Among survivors, complete litter resorption occurred in 0/24 (0%), 1/11 (9%), 1/12 (8%), 8/10 (80%), and 12/16 (75%), respectively. Maternal body weights of the 15-ppm group were significantly ($p \leq 0.05$) reduced on GD 18, mainly due to a decrease in body-weight gain within 48 h of exposure; only data for the 15-ppm group were presented graphically. Group mean placental weights and fetal body weights were significantly ($p \leq 0.05$) reduced at all concentrations to 78–93% and 73–93%, respectively, of the concurrent control. Fetuses from dams exposed at 9 ppm and 15 ppm also had significant ($p \leq 0.05$) reduction in the lengths of the mandible and long bones (group means compared with fetuses from control dams; statistical test not stated).

In a similar study (Varma et al. 1990), pregnant Swiss mice (total of 51 control and 63 MIC-exposed animals) were exposed in flow-through chambers to MIC at 9 ppm (analytical) for 3 h on GD 8. Dams (N=4–18/d)

were sacrificed on GD 10–18, and the uterine contents examined for live fetuses and postimplantation loss. Group mean values for maternal body weights, fetal weights, and placental weights were significantly ($p \leq 0.05$; Student's t-test) reduced at all postexposure periods compared with controls, and by GD 18 these parameters were 64%, 82%, and 79%, respectively, of the control levels. Fetal deaths were observed within 2 d after maternal exposure. Complete litter resorption was observed in 70% of animals. A separate experiment was conducted to determine whether fetal toxicity of MIC was due to a decrease in maternal progesterone levels. Progesterone levels were found to be significantly higher in dams that retained pregnancy than in mice that lost their litters. However, daily administration of progesterone after exposure did not decrease fetal death.

Schwetz et al. (1987) conducted a series of developmental and reproductive toxicity studies with Swiss (CD-1) mice. Exposure was whole-body in flow-through chambers. Pregnant females (N=39–44) were exposed at 0, 1, or 3 ppm for 6 h/d on GD 14–17; dams were allowed to litter for evaluation of neonatal survival. Analytical concentrations in the exposure chamber were within 10% of nominal. Exposure to MIC had no effect on maternal survival, body weight, clinical signs, or gestation length. A significant ($p<0.05$) increase in the total number of dead fetuses at birth was observed in both exposure groups (3.3% and 6.4% dead, respectively, versus 0.4% of controls). An increase in pup mortality during lactation was observed in the 3-ppm group, with the most pronounced effect during lactation days 0–4 (11.3% died versus 2.0% of controls). Pup mortalities in the 3-ppm group resulted in significantly ($p<0.05$) fewer pups per litter on lactation days 0–4. No differences in pup body weights occurred during lactation between the treated and control groups.

Schwetz et al. (1987) also evaluated 30 male and female Swiss (CD-1) mice that inhaled 0, 1, or 3 ppm 6 h/d for 4 consecutive days. Mating trials were conducted during weeks 1, 8, and 17 postexposure. This same exposure regimen was conducted with 30 males followed by mating to untreated females for 8 wk. No significant effect on body weight, demeanor, fertility, or litter size and no evidence of a dominant lethal effect was observed.

In a dominant lethal assay, 24 male Wistar rats were exposed using a whole-body protocol to MIC at 1,344 ppm for 8 min in a static exposure chamber and sequentially mated to unexposed females for 3 wk (Agarwal and Bose 1992). Total deaths among exposed males during days 1–7, 8–14, and 15–21 were 13, 5, and 3, respectively. No evidence of dominant lethality and no effects on epididymal sperm density and morphology were

observed. A transient reduction in reproductive performance of exposed males during days 1–14 postexposure was attributed to general stress.

3.4. Genotoxicity

A small but significant increase in the frequency of sister chromatid exchange (SCE) was observed in lung cells from mice exposed to MIC at 1, 3, or 6 ppm 6 h/d for 4 d; SCEs were not induced in peripheral lymphocytes (Kligerman et al. 1987). No evidence of chromosomal effects or micronuclei formation were observed in the bone marrow of mice exposed at 3, 10, or 30 ppm for 2 h (Shelby et al. 1987) or to up to 7.20 µL evaporated in a 22 L chamber for two 10-min exposures separated by 24 h (Kar et al. 1989).

MIC failed to induce mutations in any of five strains of *Salmonella* or in the *Drosophila* sex-linked recessive lethal assay following exposure of males by inhalation, feeding, or injection (Mason et al. 1987). However, dose-related increases in the number of thymidine kinase mutants were induced in L5178Y mouse lymphoma cells, and the frequencies of SCE and chromosomal aberrations increased in Chinese hamster ovary cells (Shelby et al. 1987).

Dominant lethality studies in mice have been described in Section 3.3.

3.5. Chronic Toxicity and Carcinogenicity

Groups of male and female F344 rats and B6C3F$_1$ mice were given a single 2-h exposure to MIC at 0, 1, 3, or 10 ppm and then held for 2 y (Bucher and Uraih 1989). The authors did not state whether these were analytical or nominal concentrations of MIC; however, in other work by these authors, concentrations were reported as analytical. No neoplastic lesions were observed in male or female mice or in female rats. In male rats the incidence of pheochromocytomas of the adrenal gland were 7/46, 14/46, 18/48, and 16/50, respectively, and the incidence of adenomas of pancreatic acinar cells were 0/50, 2/50, 0/49, and 6/50, respectively. The authors cautioned that although the incidence rates may indicate weak evidence for carcinogenicity and there was no evidence for dose-response, the adrenal tumor incidences were only slightly greater than the historical control mean of 22% and both tumor types are not uncommon in rats. Neither of these organs have been found to be targets for MIC.

3.6. Summary

Numerous studies have been conducted to determine the effects of MIC in animals following inhalation exposure. LC_{50} values for guinea pigs, rats, and mice (Table 3–4) show little species variability. It is interesting to note that in most studies, deaths did not occur during exposure—animals began dying within 24–48 h postexposure with another phase of deaths often occurring several days later. This pattern was similar to that in humans following the Bhopal accident in which deaths were not instantaneous but occurred after a lag period and in phases (Section 2.1).

Clinical signs of toxicity and histological lesions of the respiratory tract following exposure to MIC were consistent among all species tested. Generally, necrosis of the respiratory epithelia was followed by regeneration and intraluminal fibrosis in the bronchi. The severity of the initial lesion was concentration-dependent and the fibroplasia became more pronounced over time. In rats and guinea pigs, the histological lesions were shown to correlate with persistent decrements in pulmonary function.

Reported no-effect levels for signs of irritation included 3 ppm for 2 h in rats (Bucher and Uraih 1989; Stevens et al. 1987) and 10 ppm for 2 h in mice (Bucher and Uraih 1989). Protocols that elicited clinical signs but were experimentally derived no-effect levels for death included 8 ppm for 6 h in rats (IRDC 1964); 2.4 ppm for 6 h in rats, guinea pigs, and mice (Dodd et al. 1985, 1986); 230 ppm for 0.1 h, 35 ppm for 1 h, and 5.4 ppm for 4 h in rats (Dow Chemical 1990); and 9 ppm for 3 h in mice (Varma et al. 1988).

Developmental and reproductive toxicity has also been shown in rats and mice following inhalation exposure to MIC. Maternal toxicity was observed in both species following exposures at ≥9 ppm. Fetal body weights were decreased following a single maternal exposure at 9 ppm for 3 h in rats and at ≥2 ppm for 3 h in mice (Varma et al. 1987). Embryo-lethality was increased in rats exposed at 9 ppm for 3 h on GD 10 (Varma et al. 1990) and in mice exposed at 9 or 15 ppm for 3 h on GD 8 (Varma 1987; Varma et al. 1990). In mice, increased numbers of dead fetuses at birth occurred following maternal exposure at 1 or 3 ppm and increased pup mortality during lactation occurred following maternal exposure at 3 ppm for 6 h/da on GD 14–17 (Schwetz et al. 1987). It should be noted that the slight increases in stillbirths observed in mice following multiple inhalation exposures at 1 or 3 ppm (Schwetz et al. 1987) did not appear as increased resorptions following single exposures at 2 or 6 ppm (Varma 1987).

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

Guinea pigs inhaled ^{14}C-MIC at 0.38 to 15.2 ppm for 1–6 h and deposition in the airways was analyzed by autoradiography (Kennedy et al. 1993). Radioactivity was highest in the proximal airways; the level of radioactivity in the airway tissue was directly related to exposure concentration times duration. Radioactivity was detected throughout the entire nasal respiratory epithelial layer in a concentration-related manner. In the tracheobronchial region and in the lung, the label accumulation was at the subepithelial level extending to the terminal bronchiole but radioactivity was not detected in the alveolar region.

The uptake and distribution of ^{14}C-MIC was measured in arterial blood of guinea pigs following exposure to concentrations at 0.5–15 ppm for periods of 1–6 h (Ferguson et al. 1988). Circulating ^{14}C showed immediate uptake with clearance gradual over a period of 3 d. A similar profile was observed for urine and bile. Uptake in the upper respiratory tract passages and distribution was to all examined tissues. In pregnant mice similarly exposed to ^{14}C-MIC, the label was detected in all tissues examined, including the uterus, placenta, and fetus (Ferguson et al. 1988).

4.2. Mechanism of Toxicity

Results from human and animal studies indicate that MIC is a severe irritant to mucous membranes. Ocular irritation was the most pronounced symptom reported in human experimental studies (Kimmerle and Eben 1964; Mellon Institute 1963a, 1970). The most frequently reported symptoms among the exposed population in Bhopal, India, were burning of the eyes, coughing, respiratory distress from pulmonary congestion, watering of the eyes, nausea, vomiting, muscle weakness, and CNS involvement secondary to hypoxia (Kamat et al. 1985; Misra et al. 1987; Lorin and Kulling 1986; Andersson et al. 1988; Weill 1988; Kamat et al. 1992). Human (Varma and Guest 1993) and animal (Fowler and Dodd 1986) fatalities are attributed to pulmonary edema.

Developmental toxicity was observed in rodents following controlled exposure to MIC. The mechanism of the systemic toxicity is unknown.

Cyanide does not contribute significantly to the toxicity of MIC.

Cyanomethemoglobin was not noted in Bhopal victims (Misra et al. 1987), pulmonary lesions are not characteristic of cyanide intoxication (Varma 1989; Weill 1988), and standard thiosulfate/nitrite cyanide antidotes have not been successful in preventing deaths in animal studies (Nemery et al. 1985b; Bucher et al. 1987a; Varma et al. 1988). Finally, the time-to-death in both humans and animals was not consistent with that associated with high dose cyanide intoxication (Varma and Guest 1993).

MIC has not been shown to be a sensitizer in either humans (Ketcham 1973; Union Carbide 1966) or animals (Mellon Institute 1970). The chemical is not an effective inhibitor of cholinesterases (Brown et al. 1987).

4.3. Other Relevant Information

4.3.1. Species Variability

Comparison of LC_{50} values for the guinea pig, rat, and mouse shows species variation no greater than 2-fold. For example, 6-h LC_{50} values were 6.1, 5.4, and 12.2 ppm, respectively (Dodd et al. 1985, 1986), while 4-h values were 10.6 ppm for the guinea pig (Mellon Institute 1970) and 11– 17.5 ppm for the rat (Fait and Dodd 1981; Mellon Institute 1970). Comparison of time-to-death from a 4-h exposure in the studies of Dodd et al. (1985, 1986) and Fowler and Dodd (1986), shows that at concentrations of 15.2–36.1 ppm guinea pigs died either during exposure or 1 d postexposure, whereas rats died 1–4 d postexposure. In addition, lesions of the respiratory tract of guinea pigs were more severe than lesions observed in rats and mice. Mice and rats are generally obligate nasal breathers, whereas guinea pigs are optimal mouth breathers when exposed to respiratory irritants. Therefore, a greater amount of MIC is deposited in the lung of the guinea pig, resulting in greater damage. Als,o guinea pigs can undergo involuntary bronchoconstriction and die in response to some irritants, which may be the reason that guinea pigs appear more sensitive to MIC than rats or mice.

4.3.2. Concentration-Exposure Duration Relationship

Values scaled for the derivation of the 10- and 30-min, and 1-, 4-, and 8-h time points were calculated from the equation $C^n \times t = k$ (ten Berge et

al. 1986) where n=1. Using 7.5-, 15-, 30-, 60-, 120-, and 240-min LC_{50} values of the Mellon Institute (1970), the least-squares linear curve fit of the graph (Appendix A), log time versus log LC_{50}, resulted in the equation y=3.48−1.01x. The slope of the line, 1.0, was identified as the exponent n.

5. DATA ANALYSIS FOR AEGL-1

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or mg/m^3]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, nonsensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

5.1. Summary of Human Data Relevant to AEGL-1

Experimental data on the effects of MIC on humans were not available for the derivation of AEGL-1. While no adverse health effects were reported following inhalation exposures at 0.4 ppm for 1–5 min (Kimmerle and Eben 1964) or at 1 ppm for 1 min (Mellon Institute 1963a), ocular and upper respiratory tract irritation and lacrimation were reported after exposure at 0.5 ppm for 10 min (Mellon Institute 1970) and at 1 ppm for 10 min (Mellon Institute 1963a). Eye irritation and lacrimation, in the absence of consistent odor perception, were also reported following exposures at 2 or 5 ppm for 1–5 min (Kimmerle and Eben 1964), at 1.75 ppm for 1 min, (Mellon Institute 1970), or at 2.5 or 5.0 ppm for 1 min (Mellon Institute 1963a). Nose and throat irritation were also reported by some, but not all, of the volunteers in these experiments.

These studies, taken together, indicate that both duration of exposure and concentration of MIC contribute to the severity of MIC-induced irritation. Extrapolation from the short experimental durations to the longer AEGL time points may not be predictive of potential adverse effects. Also, it is not known at what concentration the risk for systemic toxicity, including end points of developmental toxicity, becomes a concern. The concentrations causing irritation in humans after several minutes are similar to, or higher than, the concentrations resulting in embryo and fetal lethality in

well conducted animal studies. Therefore, the human experimental exposure data were not used in derivation of AEGL-1.

5.2. Summary of Animal Data Relevant to AEGL-1

Animal data relevant to derivation of AEGL-1 were not found. Exposure concentration-duration regimens that were reported as no-effect levels for signs of irritation were similar to protocols used in other studies that caused embryolethality. Many animal studies used concentrations of MIC sufficient to result in severe damage to the respiratory system.

5.3. Derivation of AEGL-1

Methyl isocyanate has notoriously poor warning properties (AIHA 1989), and AEGL-1 values were not derived. Although human and animal data were available for irritation levels, the ambient air concentrations associated with MIC sensory irritation may be greater than those associated with systemic toxicity. However, it should be noted that exposures to MIC at concentrations below those used to calculate AEGL-1 might be associated with systemic toxicity.

6. DATA ANALYSIS FOR AEGL-2

AEGL-2 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.

6.1. Summary of Human Data Relevant to AEGL-2

Human data relevant to AEGL-2 derivation are limited to studies that used very short exposure durations. Among volunteers exposed at 1 ppm for 10 min, 7/7 had eye irritation and tears by 4 and 5 min, respectively, and nose and throat irritation were reported by 3/7 after 9 min (Mellon Institute 1963a). Eye irritation and tearing were also reported for individuals ex

posed at 2 or 4 ppm for 1–5 min (Kimmerle and Eben 1964) and at 0.5 ppm for 10 min (Mellon Institute 1970).

6.2. Summary of Animal Data Relevant to AEGL-2

Pregnant Swiss-Webster mice were exposed to analytically monitored concentrations of MIC at 0, 2, 6, 9, and 15 ppm for 3 h on GD 8 (Varma 1987). Placental weights and fetal body weights were significantly reduced at all MIC concentrations. Maternal toxicity was evident as deaths of two of the 11 treated dams in the 9- and 15-ppm groups and decreased maternal body weights at 15 ppm. Exposures at 9 or 15 ppm also resulted in a significant increase in complete litter resorption among surviving dams, and fetuses with significant reductions in the lengths of the mandible and long bones. Decreased maternal body weight and increased resorptions also occurred in rats exposed at 9 ppm for 3 h on GD 10 (Varma et al. 1990). Taken together these studies indicate that in pregnant rats and mice exposed once to MIC by inhalation, significant reductions in fetal body weight occur in the absence of maternal toxicity but embryo and fetal lethality occur at concentrations that result in overt maternal toxicity.

An increase in cardiac arrhythmias occurred in rats 4 mo after a 2-h exposure at 3 ppm (Tepper et al. 1987).

No effects on survival, body weights, or lung histopathology were noted in rats 2 y after exposure at 1 or 3 ppm for 2 h (Bucher and Uraih 1989).

6.3. Derivation of AEGL-2

Animal data were used for derivation of AEGL-2 values. The exposure of mice at 2 ppm for 3 h on GD 8 was a LOEL for lower fetal body weights in the absence of maternal toxicity (Varma 1987), and the exposure of rats at 3 ppm for 2 h was a LOEL for cardiac arrhythmias (Tepper et al. 1987). These exposure concentration and duration scenarios yield identical AEGL-2 values when used for derivation. Details of the calculations are shown in Appendix B. Values scaled for the derivation of the 10- and 30-min and 1-, 4-, and 8-h time points were calculated from the equation $C^n \times t = k$ (ten Berge et al. 1986) where n=1 as discussed in Section 4.4.2. The experimental concentrations were reduced by a factor of 3 to estimate a threshold

for effects on fetal body weight and cardiac arrhythmias. A total uncertainty factor of 30 was applied, including 3 for interspecies variation, because similar results for developmental toxicity have been obtained in both rats and mice, and 10 for intraspecies variation, because the mechanism of action for developmental toxicity is unknown. Proposed AEGL-2 values are presented in Table 3–5.

TABLE 3–5 AEGL-2 Values for Methyl Isocyanate (ppm [mg/m3])

10 min	30 min	1 h	4 h	8 h
0.40 (0.94)	0.13 (0.32)	0.067 (0.16)	0.017 (0.034)	0.008 (0.02)

7. DATA ANALYSIS FOR AEGL-3

AEGL-3 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

7.1. Summary of Human Data Relevant to AEGL-3

Human data relevant to derivation of AEGL-3 were not found. Concentrations that resulted in death, spontaneous abortion, or infant death during the accidental release in Bhopal, India, are unknown.

7.2. Summary of Animal Data Relevant to AEGL-3

Schwetz et al. (1987) exposed pregnant mice at 0, 1, or 3 ppm for 6 h/d on GD 14–17. Dams were allowed to litter for evaluation of neonatal survival. No maternal toxicity was observed at either exposure concentration. A concentration-related increase in the number of dead fetuses at birth was observed in both exposure groups and an increase in pup mortality during lactation was observed in the 3-ppm group. Pup mortalities in the 3-ppm group resulted in significantly ($p<0.05$) fewer pups per litter on lactation days 0–4. No differences in pup body weights occurred during lactation between the treated and control groups. It should be noted that the slight

increases in stillbirths observed in mice following multiple inhalation exposures at 1 or 3 ppm (Schwetz et al. 1987) did not appear as increased resorptions following single exposures at 2 or 6 ppm (Varma 1987).

Pregnant rats (Varma et al. 1990) and mice (Varma 1987) exposed at 9 ppm for 3 h on GD 10 or 8, respectively, had increased resorptions and decreased maternal body weights, fetal body weights, and placental weights.

7.3. Derivation of AEGL-3

The neonatal survival study with mice by Schwetz et al. (1987) was used for derivation of AEGL-3 values. The study was well conducted and included sufficient concentration-response data. Although the exposures were repeated on 4 consecutive days, the exposure to the fetus is considered similar to a single exposure because the stage of development and potential susceptibility changes daily throughout gestation. In addition, the lower pup survival seen experimentally following repeated maternal exposure, is the same end point as fetal and infant death in humans observed following the Bhopal accident. Therefore, the NAC/AEGL FACA committee considered this study appropriate for derivation of AEGL-3 values.

The 6-h exposure at 1 ppm was used to derive AEGL-3 values using the equation $C^n \times t = k$ where n=1 as described in Section 4.4.2. This concentration was a NOEL for pup survival during lactation. A total uncertainty factor (UF) of 30 was applied, including 3 for interspecies variation, because similar developmental toxicity results have been obtained in both rats and mice, and 10 for intraspecies variation, because the mechanism of action for developmental toxicity is unknown. According to the standing operating procedures of the NAC/AEGL FACA committee, 10-min values are not to be scaled from an experimental exposure time of ≥4 h. However, because n was derived from exposures ranging from 7.5 to 240 min, extrapolation from 6 h to the 10-min AEGL-3 value is valid in this instance. Details of the calculations are shown in Appendix B. AEGL-3 values for MIC are listed in Table 3–6.

Exposures of 9 ppm for 3 h from the developmental toxicity studies in rats (Varma et al. 1990) and mice (Varma 1987) could also be used to derive AEGL-3 values. Derivations based on those data, using the UF of 30, result in AEGL-3 values of 5.4, 1.8, 0.9, 0.23, and 0.11 ppm. They would be less conservative AEGL-3 values that might not be protective of neonates.

TABLE 3–6 AEGL-3 Values for Methyl Isocyanate [ppm (mg/m3)]

10 min	30 min	1 h	4 h	8 h
1.2 (2.8)	0.40 (0.95)	0.20 (0.47)	0.05 (0.12)	0.025 (0.06)

8. SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity End Points

The derived AEGL values for various levels of effects and durations of exposure are summarized in Table 3–7. Data were not available for derivation of AEGL-1; the irritation threshold may be above the level of concern for systemic effects. However, it should be noted that exposures to MIC at concentrations below those used to calculate AEGL-1 might be associated with systemic toxicity. AEGL-2 is based on lower fetal body weights in mice and cardiac arrhythmias in rats. AEGL-3 is based on decreased neonatal survival in mice. Derivation summaries, including key studies and end points for each AEGL level, are given in Appendix C.

A useful way to evaluate the AEGL values in context of existing empirical data is presented in Figure D-1 of Appendix D. For this plot, the toxic response was placed into severity categories. The severity categories fit into definitions of the AEGL health effects: no effects, discomfort, disabling, partial lethality, and lethality (100% mortality). The effects that place an experimental result into a particular category vary according to the spectrum of data available on a specific chemical and the effects from exposure to that chemical. The concentrations often span a number of orders of magnitude, especially when human data exist, and are therefore plotted on a log scale. The graph in Figure D-1 plots the AEGL values for MIC along with the existing acute human and animal toxicity data for MIC in terms of the categories assigned to them. From this plot, it is apparent that the AEGL values are below any exposure concentration in animals resulting in adverse effects (including developmental toxicity) and should therefore be protective of human health.

8.2. Comparison with Other Standards and Criteria

Existing guideline exposure levels for MIC are listed in Table 3–8. All

of the standards listed have a skin notation. The American Industrial Hygiene Association (AIHA 2000) derived an ERPG-1 based on the belief that nearly all individuals could be exposed for 1 h at 0.025 ppm without experiencing or developing adverse effects more serious than mild irritation or perception of a clearly objectionable odor. The 1-h AEGL-2 and AEGL-3 values are substantially less than the ERPG-2 and ERPG-3 values because of the different end points setting the levels. The ERPG-2 was based on the RD_{50} of 1.3 ppm, the increased spontaneous abortion in Bhopal, and the fact that 1 ppm was a NOAEL in rats exposed 6 h/d for 4 or 8 d; the AGEL-2 was based on reduced fetal body weight. The ERPG-3 was based on nonlethal acute inhalation data in mice and guinea pigs (5.4 ppm for 6 h and 10.6 ppm for 2 h, respectively) and the 1-h LC_{50} of 41.3 ppm in rats; in contrast, the AEGL-3 was based on fetotoxicity in rodents following maternal exposure during gestation. The AEGL-3 was of particular concern given the reports of spontaneous abortion among women pregnant during the Bhopal accident.

TABLE 3–7 Summary of AEGL Values (ppm [mg/m3])

	Exposure Duration				
Classification	10 min	30 min	1 h	4 h	8 h
AEGL-1[a] (Nondisabling)	NR	NR	NR	NR	NR
AEGL-2 (Disabling)	0.40 (0.94)	0.13 (0.32)	0.067 (0.16)	0.017 (0.034)	0.008 (0.02)
AEGL-3 (Lethal)	1.2 (2.8)	0.40 (0.95)	0.20 (0.47)	0.05 (0.12)	0.025 (0.06)

[a]Exposure to MIC at concentrations below those used to calculate AEGL-1 may be associated with systemic toxicity.
Abbreviation: NR, not recommended.

The NIOSH IDLH (NIOSH 1996) is greater than the 30-min AEGL-3. NIOSH based their IDLH on acute inhalation toxicity data in humans from Kimmerle and Eben (1964) and from the Mellon Institute (1963a).

The Dutch MAC (2000) of 0.02 ppm is equal to the TWA values of NIOSH, OSHA, and ACGIH, but the German MAK (2000) of 0.01 ppm is less than these. The MAK excursion peak category is I (with an excursion factor of 1) for a substance for which local irritant effects determine the MAK value.

TABLE 3–8 Extant Standards and Guidelines for Methyl Isocyanate Exposure Duration

	Exposure Duration				
Guideline	10 min	30 min	1 h	4 h	8 h
AEGL-1	NR	NR	NR	NR	NR
AEGL-2	0.40 ppm	0.13 ppm	0.067 ppm	0.017 ppm	0.008 ppm
AEGL-3	1.2 ppm	0.40 ppm	0.20 ppm	0.05 ppm	0.025 ppm
ERPG-1 (AIHA)[a]			0.025 ppm		
ERPG-2 (AIHA)	0.50 ppm				
ERPG-3 (AIHA)	5.0 ppm				
PEL-TWA (OSHA)[b]			0.02 ppm		
PEL-STEL (OSHA)[c]	0.02 ppm				
IDLH (NIOSH)[d]		3 ppm			
REL-TWA (NIOSH)[e]		0.02 ppm			
TLV-TWA (ACGIH)[f]		0.02 ppm			
MAK (Germany)[g]		0.01 ppm			
MAK Peak Limit (Germany)[h]		Category I (excursion factor 1)			
MAC (The Netherlands)[i]					0.02 ppm

[a]ERPG (emergency response planning guidelines) (AIHA 1995). The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing other than mild, transient adverse health effects or without perceiving a clearly defined objectionable odor. The ERPG-1 for MIC is based on belief that nearly all individuals could be exposed to this concentration without experiencing or developing serious effects. The ERPG-2 is the maximum airborne concentration below which it is believed nearly

all individuals could be exposed for up to 1 h without experiencing or developing irreversible or other serious health effects or symptoms that could impair an individual's ability to take protection action. The ERPG-2 for MIC is based on the RD_{50} of 1.3 ppm, reproductive effects noted in Bhopal, India, and the fact that 1 ppm was a NOAEL in rats exposed for 6 h/d for 4 or 8 d. The ERPG-3 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing or developing life-threatening health effects. The ERPG-3 for MIC is based on acute inhalation data in which exposure of 5.4 ppm for 6 h were nonlethal in mice, 10.6 ppm for 2 h was nonlethal in guinea pigs, and the 1-h LC_{50} for rats is 41.3 ppm.

[b]OSHA PEL-TWA (permissible exposure limit-time-weighted average) (OSHA 1995) is defined analogous to the ACGIH-TLV-TWA, but is for exposures of no more than 10 h/d, 40 h/wk.

[c]OSHA PEL-STEL (permissible exposure limit-short-term exposure limit) (OSHA 1995) is defined analogous to the ACGIH-TLV-STEL.

[d]IDLH (immediately dangerous to life and health) (NIOSH 1996) represents the maximum concentration from which one could escape within 30 min without any escape-impairing symptoms or any irreversible hearth effects. The IDLH for MIC is based on acute inhalation toxicity data in humans.

[e]NIOSH REL-TWA (recommended exposure limit-time-weighted average) (NIOSH 1997) is defined analogous to the ACGIH TLV-STEL.

[f]ACGIH TLV-TWA (American Conference of Governmental Industrial Hygienists Threshold Limit Value-time-weighted average) (ACGIH 1991, 2001) is the time-weighted average concentration for a normal 8-h workday and a 40-h workweek to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

[g]MAK (Maximale Argeitsplatzkonzentration [Maximum Workplace Concentration]) (Deutsche Forschungsgemeinschaft [German Research Association] 2000) is defined analogous to the ACGIH-TLV-TWA.

[h]MAK Spitzenbegrenzung (Peak Limit I) (German Research Association 2000) constitutes the maximum average concentration to which workers can be exposed for a period up to 15 min with no more than four exposure periods per work shift and at least 1 h between exposure peaks; total exposure may not exceed 8-h MAK.

[i]MAC (Maximaal Aanvaaarde Concentratie [Maximal Accepted Concentration]) (SDU Uitgevers [under the auspices of the Ministry of Social Affairs and Employment], The Hague, The Netherlands 2000) is defined analogous to the ACGIH-TLV-TWA.

Abbreviation: NR, not reported.

8.3. Data Adequacy and Research Needs

Mainly qualitative data are available regarding acute exposures of

humans to MIC. Although extensive research has been published describing the immediate and long-term effects on humans following the Bhopal disaster, exact exposure conditions are lacking. Experimental studies in humans are of extremely short duration (i.e., minutes) and do not adequately define a dose-response. Animal data have shown concentration-dependent effects including irritation and histological lesions of the respiratory tract, developmental toxicity, and neonatal and adult lethality. Because the nonlethal and lethal effects in humans and animals are qualitatively similar, the animal data were considered relevant and appropriate for development of AEGL values as described in the standing operating procedures of the National Advisory Committee for AEGLs.

The most notable data deficiencies were the absence of quantitative human exposure data, the absence of a well-defined exposure-response curve for the toxic effects in animals, an understanding of the mechanism of action for systemic effects, and an understanding of individual variability in the toxic response to MIC.

Critical research needs include defining thresholds for effects and how these thresholds may vary with exposure concentration and duration. Such data would be valuable for affirming the AEGL values. In addition, the mechanism of systemic toxicity is unknown, and therefore, research into the mechanism(s) of systemic toxicity and the relationship to irritation effects would be instrumental in reducing uncertainties in quantitative health risk issues.

9. REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 1991. Methyl Isocyanate. Pp. 1022–1024 in Documentation of the Threshold Limit Values and Biological Exposure Indicies, 6th Ed. ACGIH, Cincinnati, OH.

ACGIH (American Conference of Governmental Industrial Hygienists). 2001. 2001 TLVs and BEIs Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices, ACGIH, Cincinnati, OH. 41 pp.

Agarwal, D.K., and M.Bose. 1992. Inhalation toxicity of methylisocyanate: Assessment of germ cell mutagenicity and reproductive effects in rats. In. J. Exp. Biol. 30:504–508.

AIHA (American Industrial Hygiene Association). 1989. Table 5.3 Reported odor thresholds from all code sources. In: Odor Thresholds for Chemicals with Established Occupational Health Standards. AIHA, Fairfax, VA. 68 pp.

AIHA (American Industrial Hygiene Association). 1995. The AIHA 1995 Emer

gency Response Planning Guidelines and Workplace Environmental Exposure Level Guides Handbook. AIHA, Fairfax, VA. 19 pp.

Alarie, Y., J.S.Ferguson, M.F.Stock, D.A.Weyel, and M.Schaper. 1987. Sensory and pulmonary irritation of methyl isocyanate in mice and pulmonary irritation and possible cyanidelike effects of methyl isocyanate in guinea pigs. Environ. Health Perspect. 72:159–167.

Andersson, N., M.K.Muir, and V.Mehra. 1984. Bhopal eye. Lancet 2:1481.

Andersson, N., M.K.Muir, A.G.Salmon, C.J. Wells, R.B.Brown, C.J.Purnell, P.C.Mittal, and V.Mehra. 1985. Bhopal disaster: Eye follow-up and analytical chemistry. Lancet 1:761–762.

Andersson, N., M.K.Muir, V.Mehra, and A.G.Salmon. 1988. Exposure and response to methyl isocyanate: Results of a community based survey in Bhopal. Br. J. Ind. Med. 45:469–475.

Andersson, N., M.K.Ajwani, S.Mahashabde, M.K.Tiwari, M.K.Muir, V.Mehra, K.Ashiru, and C.D.Mackenzie. 1990. Delayed eye and other consequences from exposure to methyl isocyanate: 93% follow up of exposed and unexposed cohorts in Bhopal . Br. J. Ind. Med. 47:553–558.

Arbuckle, T.E., and L.E.Sever. 1998. Pesticide exposures and fetal death: A review of the epidemiologic literature. Crit. Rev. Toxicol. 28:229–270.

Boorman, G.A., R.Brown, B.N.Gupta, L.C.Uraih, and J.R.Bucher. 1987a. Pathological changes following acute methyl isocyanate inhalation and recovery in B6C3F1 mice. Toxicol. Appl. Pharmacol. 87:446–456.

Boorman, G.A., L.C.Uraih, B.N.Gupta, and J.R.Bucher. 1987b. Two-hour methyl isocyanate inhalation and 90-day recovery study in B6C3F1 mice. Environ. Health Perspect. 72:63–69.

Brown, W.E., A.H.Green, T.E.Cedel, and J.Cairns. 1987. Biochemistry of protein-isocyanate interactions: A comparison of the effects of aryl vs. alkyl isocyanates. Environ. Health Perspect. 72:5–11.

Bucher, J.R., B.N.Gupta, B.Adkins, Jr., M.Thompson, C.W.Jameson, J.E. Thigpen, and B.A.Schwetz. 1987a. Toxicity of inhaled methyl isocyanate in F344/N rats and B6C3F1 mice. I. Acute exposure and recovery studies. Environ. Health Perspect. 72:53–61.

Bucher, J.R., G.A.Boorman, B.N.Gupta, L.C.Uraih, L.B.Hall, and S.A. Stefanski. 1987b. Two-hour methyl isocyanate inhalation exposure and 91-day recovery: A preliminary description of pathologic changes in F344 rats. Environ. Health Perspect. 72:71–75.

Bucher, J.R., and L.C.Uraih. 1989. Carcinogenicity and pulmonary pathology associated with a single 2-hour inhalation exposure of laboratory rodents to methyl isocyanate. J. Natl. Cancer Inst. 81:1586–1587.

Budavari, S., M.J.O'Neil, A.Smith, P.E.Heckelman, and J.F.Kinneary, eds. 1996. The Merck Index, 11th Ed. Rahway, NJ: Merck and Co., Inc. 1,039 pp.

Cullinan, P., S.Acquilla, and V.R.Dhara. 1997. Respiratory morbidity 10 years after the Union Carbide gas leak at Bhopal: A cross sectional survey. Br. Med. J. 314:338–343.

Dinsdale, D., B.Nemery, and S.Sparrow. 1987. Ultrastructural changes in the respiratory tract of rats following methyl isocyanate inhalation. Arch. Toxicol. 59:385–390.

Dodd, D.E., E.H.Fowler, W.M.Snellings, I.M.Pritts, and R.L.Baron. 1985. Acute inhalation studies with methyl isocyanate. Bushy Run Research Center. Sponsored by Union Carbide Corporation. EPA/OTS; Doc #88–8600039. 100 pp.

Dodd, D.E., E.H.Fowler, W.M.Snellings, I.M.Pritts, and R.L.Baron. 1986. Acute inhalation studies with methyl isocyanate vapor. I. Methodology and LC50 determinations in guinea pigs, rats, and mice. Fundam. Appl. Toxicol. 6:747–755.

Dodd, D.E., F.R.Frank, E.H.Fowler, C.M.Troup, and R.M.Milton. 1987. Biological effects of short-term, high-concentration exposure to methyl isocyanate. I. Study objectives and inhalation exposure design. Environ. Health Perspect. 72:13–19.

Dow Chemical. 1990. The Dow Chemical Company. Results of single exposure of rats and rabbits to the vapors of methyl isocyanate. Dow report identification number D004632. EPA/OTS; Doc #86–910000362S. 7 pp.

Dutta, K.K., G.S.D.Gupta, A.Mishra, A.Joshi, G.S.Tandon, and P.K.Ray. 1988. Inhalation toxicity studies of methyl isocyanate (MIC) in rats: Part I—Pulmonary pathology and genotoxicity evaluation. Ind. J. Exp. Biol. 26:177–182.

Dwivedi, P.C., J.K.Raizada, V.K.Saini, and P.C.Mittal. 1985. Ocular lesions following methyl isocyanate contamination: The Bhopal experience. Arch. Ophthalmol. 103:1627.

Dwivedi, P.D., A.Mishra, G.S.D.Gupta, K.K.Dutta, S.N.Das, and P.K.Ray. 1988. Inhalation toxicity studies of methyl isocyanate (MIC) in rats: Part IV— Immunologic response of rats one week after exposure: Effect on body and organ weights, phagocytic and DTH response. Ind. J. Exp. Biol. 26:191–194.

Eastman Kodak. 1966. Eastman Kodak Company. Toxicity and health hazard summary. Lab. No. 63–832. EPA/OTS; Doc #86–910000056. 6 pp.

EPA (U.S. Environmental Protection Agency). 1986. Health and Environmental Effects Profile for Methyl Isocyanate. Office of Health and Environmental Assessment, EPA, Cincinnati, OH. 52 pp.

Fait, D.W. and D.E.Dodd. 1981. Methyl isocyanate four-hour acute LC_{50} inhalation study on rats and guinea pigs. Bushy Run Research Center. Sponsored by Union Carbide Corporation. EPA/OTS; Doc #86–920000613. 6 pp.

Fedde, M.R., D.E.Dodd, C.M.Troup, and E.H.Fowler. 1987. Biological effects of short-term, high-concentration exposure to methyl isocyanate. III. Influence on gas exchange in the guinea pig lung. Environ. Health Perspect. 72:189–195.

Ferguson, J.S., and Y.Alarie. 1991. Long term pulmonary impairment following a single exposure to methyl isocyanate. Toxicol. Appl. Pharmacol. 107:253– 268.

Ferguson, J.S., M.Schaper, M.F.Stock, D.A.Weyel, and Y.Alarie. 1986. Sen

sory and pulmonary irritation with exposure to methyl isocyanate. Toxicol. Appl. Pharmacol. 82:329–335.

Ferguson, J.S., A.L.Kennedy, M.F.Stock, W.E.Brown, and Y.Alarie. 1988. Uptake and distribution of ^{14}C during and following exposure to [^{14}C]methyl isocyanate. Toxicol. Appl. Pharmacol. 94:104–117.

Fowler, E.H., and D.E.Dodd. 1985. Respiratory tract changes in guinea pigs, rats, and mice following acute exposure to methyl isocyanate vapor. Bushy Run Research Center. Sponsored by Union Carbide Corporation. EPA/OTS; Doc #88–8600039. 100 pp.

Fowler, E.H., and D.E.Dodd. 1986. Acute inhalation studies with methyl isocyanate vapor. II. Respiratory tract changes in guinea pigs, rats, and mice. Fundam. Appl. Toxicol. 6:756–771.

Fowler, E.H., D.E.Dodd, and C.M.Troup. 1987. Biological effects of short-term, high-concentration exposure to methyl isocyanate. V. Morphologic evaluation of rat and guinea pig lungs. Environ. Health Perspect. 72:39–44.

Gassert, T., C.MacKenzie, M.Kerr Muir, N.Andersson, and A.G.Salmon. 1986. Long term pathology of lung, eye, and other organs following acute exposure of rats to methyl isocyanate. Lancet 2:1403.

German Research Association (Deutsche Forschungsgemeinschaft). 2000. List of MAK and BAT Values, 2000. Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Report No. 36. Weinheim, Federal Republic of Germany: Wiley VCH.

Ghosh, B.B., S.Sengupta, A.Roy, S.Maity, S.Ghosh, G.Talukder, and A. Sharma. 1990. Cytogenetic studies in human populations exposed to gas leak at Bhopal, India. Environ. Health Perspect. 86:323–326.

Goswami, H.K. 1986. Cytogenetic effects of methyl isocyanate exposure in Bhopal. Hum. Genet. 74:81–84.

Gupta, B.N., S.A.Stefanski, J.R.Bucher, and L.B.Hall. 1987. Effect of methyl isocyanate (MIC) gas on the eyes of Fischer 344 rats. Environ. Health Perspect. 72:105–108.

Gupta, G.S.D., F.N.J.Jaffery, K.K.Dutta, P.N.Viswanathan, A.Mishra, and P.K. Ray. 1988. Inhalation toxicity studies of methyl isocyanate (MIC) in rats: Part II—Biochemical studies of hemolysates, lactate dehydrogenase, isoenzymes and lipid peroxidation. Ind. J. Exp. Biol. 26:183–186.

Gupta, G.S.D., J.L.Kaw, S.H.N.Naqvi, R.Dixit, and P.K.Ray. 1991. Inhalation toxicity of methyl isocyanate: biochemical and cytological profile of bronchoalveolar lavage fluid in rats. J. Appl. Toxicol. 11:157–160.

Hartung, R. 1994. Cyanides and Nitriles. In: Patty's Industrial Hygiene and Toxicology, 4th Ed. G.D.Clayton and F.E.Clayton, eds. pp. 3161–3172. New York: John Wiley & Sons, Inc.

Irani, S.F., and A.A.Mahashur. 1986. A survey of Bhopal children affected by methyl isocyanate gas. J. Postgrad. Med. 32:195–198.

IRDC (International Research and Development Corporation). 1964. Six-hour

acute inhalation toxicity study in rats. Report 203–001. Sponsored by The Carwin Co. EPA/OTS; Doc #86–870002225. 6 pp.

James, J.T., L.C.Buettner, and S.S.Hsu. 1987. Sensory irritation of methylisocyanate vapor. J. Appl. Toxicol. 7:147–148.

Jeevaratnam, K., R.Vijayaraghavan, M.P.Kaushik, and C.S.Vaidyanathan. 1990. Acute toxicity of methyl isocyanate in mammals. II. Induction of hyperglycemia, lactic acidosis, uremia, and hypothermia in rats. Arch. Environ. Contam. Toxicol. 19:314–318.

Jeevaratnam, K., and S.Sriramachari. 1994. Comparative toxicity of methyl isocyanate and its hydrolytic derivatives in rats. I. Pulmonary histopathology in the acute phase. Arch. Toxicol. 69:39–44.

Kamat, S.R., A.A.Mahashur, A.K.B.Tiwaris, V.Potdar, M.Gaur, V.P.Kolhatkar, P.Vaidya, D.Parmar, R.Rupwate, T.S.Chatterjee, K.Jain, M.D.Kelkar, and S.G.Kinare. 1985. Early observations on pulmonary changes and clinical morbidity due to the isocyanate gas leak at Bhopal. J. Postgrad. Med. 31:63– 72.

Kamat, S.R., M.H.Patel, P.V.Pradhan, S.P.Taskar, P.R.Vaidya, V.P.Kolhatkar, J.P.Gopalani, J.P.Chandarana, N.Dalal, and M.Naik. 1992. Sequential respiratory, psychologic, and immunologic studies in relation to methyl isocyanate exposure over two years with model development. Environ. Health Perspect. 97:241–253.

Kar, R.N., K.A.Khan, and N.Sethi. 1989. Genotoxicity studies on mice after short term inhalation exposure to methyl isocyanate. Cytobios 59:167–176.

Karlsson, E., N.Karlsson, G.Lindberg, B.Lindgren, and S.Winter. 1985. The Bhopal catastrophe—consequences of a liquefied gas discharge. National Defense Research Institute, Sweden. NTIS ISSN 0347–2124.

Karol, M.H., S.Taskar, S.Gangal, B.F.Rubanoff, and S.R.Kamat. 1987. The antibody response to methyl isocyanate: Experimental and clinical findings. Environ. Health Perspect. 72:169–175.

Kennedy, A.L., G.Singh, Y.Alarie, and W.E.Brown. 1993. Autoradiographic analyses of guinea pig airway tissues following inhalation exposure to ^{14}C-labeled methyl isocyanate. Fund. Appl. Toxicol. 20:57–67.

Khurrum, M.A., and S.H.Ahmad. 1987. Long term follow up of ocular lesion of methyl-isocyanate gas disaster in Bhopal. Ind. J. Ophthalmol. 35:136–137.

Kimmerle, G., and A.Eben. 1964. On the toxicity of methyl isocyanate and its quantitative determination in air [in German]. Arch. Toxikol. 20:235–241.

Kirk-Othmer. 1994. Encyclopedia of Chemical Technology, 4th Ed., pp. 435–436. New York: John Wiley & Sons.

Kligerman, A.D., J.A.Campbell, G.L.Erexson, J.W.Allen, and M.D.Shelby. 1987. Sister chromatid exchange analysis in lung and peripheral blood lymphocytes of mice exposed to methyl isocyanate by inhalation. Environ. Mutagen. 9:29–36.

Kolb, W.P., J.R.Savary, C.M.Troup, D.E.Dodd, and J.D.Tamerius. 1987. Biological effects of short-term, high-concentration exposure to methyl isocyanate.

VI. In vitro and in vivo complement activation studies. Environ. Health Perspect. 72:189–195.

Lorin, H.G., and P.E.J.Kulling. 1986. The Bhopal tragedy—what has Swedish disaster medicine planning learned from it? J. Emerg. Med. 4:311–316.

Luster, M.I., A.N.Tucker, D.R.Germolec, M.T.Silver, P.T.Thomas, S.J.Vore, and J.R.Bucher. 1986. Immunotoxicity studies in mice exposed to methyl isocyanate. Toxicol. Appl. Pharmacol. 86:140–144.

Maginness, L.A., J.M.Szewczak, and C.M.Troup. 1987. Biological effects of short-term, high-concentration exposure to methyl isocyanate. IV. Influence on the oxygen-binding properties of guinea pig blood. Environ. Health Perspect. 72:35–38.

ManTech Environmental (ManTech Environmental Technology, Inc). 1992. Acute 1-hour inhalation toxicity study of methyl isocyanate in rats. Project No. 6030– 003. Sponsored by Rhone-Poulenc Ag Company. EPA/OTS; Doc #86– 920000962. 25 pp.

Maskati, Q.B. 1986. Ophthalmic survey of Bhopal victims 104 days after the tragedy. J. Postgrad. Med. 32:199–202.

Mason, J.M., E.Zeiger, S.Haworth, J.Ivett, and R.Valencia. 1987. Genotoxicity studies of methyl isocyanate in *Salmonella, Drosophila,* and cultured Chinese hamster ovary cells. Environ. Mutagen. 9:19–28.

Mellon Institute. 1963a. The feasibility of using methyl isocyanate as a warning agent in liquid carbon monoxide. Report 26–23. Sponsored by Union Carbide Chemicals, Co. EPA/OTS; Doc #86–910000271. 5 pp.

Mellon Institute. 1963b. Range finding tests on methyl isocyanate. Report 26–75. Sponsored by Union Carbide Chemicals, Co. EPA/OTS; Doc #86–910000658. 6 pp.

Mellon Institute. 1966. Miscellaneous acute toxicity data. Report 28–141. Sponsored by Union Carbide Chemicals, Co. EPA/OTS; Doc #86–910000267. 2 pp.

Mellon Institute. 1970. Acute inhalation toxicity, human response to low concentrations, guinea pig sensitization, and cross sensitization to other isocyanates. Report 33–19. Sponsored by Union Carbide Chemicals, Co. EPA/OTS; Doc #86–910000268. 8 pp.

Ministry of Social Affairs and Employment (SDU Uitgevers). 2000. Nationale MAC (Maximum Allowable Concentration) List, 2000. The Hague, The Netherlands.

Mishra, A., G.S.D.Gupta, V.S.Kumar, K.K.Dutta, and P.K.Ray. 1988. Inhalation toxicity studies of methyl isocyanate (MIC) in rats: Part III—Studies on biotransformation enzymes. Ind. J. Exp. Biol. 26:187–190.

Misra, N.P., R.Pathak, K.J.B.S.Gaur, S.C.Jain, S.S.Yesikar, P.C.Manoria, K.N. Sharma, B.M.Tripathi, B.S.Asthana, H.H.Trivedi, V.K.Sharma, Y. Malhotra, A.Verma, D.K.Bhargava, and G.Batni. 1987. Clinical profile of gas leak victims in acute phase after Bhopal episode. Indian J. Med. Res. 86:11–19.

Misra, U.K. and J.Kalita. 1997. A study of cognitive functions in methyl-isocyanate victims one year after Bhopal accident. Neurotoxicol. 18:381–386.

Mitsumori, K., G.A.Boorman, B.N.Gupta, and J.R.Bucher. 1987. Four-day repeated inhalation and recovery study of methyl isocyanate in F344 rats and B6C3F1 mice. Fund. Appl. Toxicol. 9:480–495.

Naik, S.R., V.N.Acharya, R.A.Bhalerao, S.S.Kowli, H.Nazareth, A.A.Mahashur, S.Shah, A.V.Potnis, and A.C.Mehta. 1986. Medical survey of methyl isocyanate gas affected population of Bhopal. Part 1: General medical observations 15 weeks following exposure. J. Postgrad. Med. 32:175–184.

Nemery, B., S.Dinsdale, S.Sparrow, and D.E.Ray. 1985a. Effects of methyl isocyanate on the respiratory tract of rats. Br. J. Ind. Med. 42:799–805.

Nemery, B., S.Sparrow, and D.Dinsdale. 1985b. Methyl isocyanate: Thiosulphate does not protect. Lancet 2:1245–1246.

NIOSH (National Institute for Occupational Safety and Health). 1996. Methyl Isocyanate. Documentation for Immediately Dangerous to Life or Health Concentrations (IDLHs). NIOSH, Cincinnati, OH. Last updated 8/16/96.

NIOSH (National Institute for Occupational Safety and Health). 1997. NIOSH Pocket Guide to Chemical Hazards. NIOSH, Cincinnati, OH.

NRC (National Research Council). 1993. Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances. Washington, DC: National Academy Press.

NRC (National Research Council). 2001. Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Chemicals. Washington, DC: National Academy Press.

OSHA (Occupational Safety and Health Administration). 1995. Table Z-1. Limits for Air Contaminants. CFR 29 Part 1910, p. 14.

Pant, S.C., R.K.Srivastava, and R.Vijayaraghavan. 1987. Histomorphologic changes induced by methyl isocyanate in lungs of rats and rabbits. Bull. Environ. Contam. Toxicol. 38:876–881.

Raizada, J.K., and P.C.Dwivedi. 1987. Chronic ocular lesions in Bhopal gas tragedy. Ind. J. Ophthalmol. 35:453–455.

Salmon, A.G., M.Kerr Muir, and N.Andersson. 1985. Acute toxicity of methyl isocyanate: A preliminary study of the dose response for eye and other effects. Br. J. Ind. Med. 42:795–798.

Saxena, A.K., K.P.Singh, K.K.Dutta, G.S.D.Gupta, S.L.Nagale, A.Mishra, S.I.A.Zaidi, and P.K.Ray. 1988. Inhalation toxicity studies of methyl isocyanate (MIC) in rats: Part V—Immunologic response of rats two weeks after exposure: Phagocytic response, endotoxin susceptibility, local lung immunity, mitogenic and DTH response. Ind. J. Exp. Biol. 26:195–200.

Schwetz, B.A., B.Adkins, Jr., M.Harris, M.Moorman, and R.Sloane. 1987. Methyl isocyanate: Reproductive and developmental toxicology studies in Swiss mice. Environ. Health Perspect. 72:149–152.

Sethi, N., R.Dayal, and R.K.Singh. 1989. Acute and subacute toxicity study of

inhaled methyl isocyanate in Charles Foster rats. Ecotoxicol. Environ. Safety 18:68–74.

Shelby, M.D., J.W.Allen, W.J.Caspary, S.Haworth, J.Ivett, A.Kligerman, C.A. Luke, J.M.Mason, B.Myhr, R.R.Tice, R.Valencia, and E.Zeiger. 1987. Results of in vitro and in vivo genetic toxicity tests on methyl isocyanate. Environ. Health Perspect. 72:183–187.

Shepard, T.H. (Ed.) 1995. Catalog of Teratogenic Agents, 8th Ed. Baltimore: The Johns Hopkins University Press. 274 pp.

Singh, R.K., R.Dayal, A.K.Srivastava, and N.Sethi. 1994. Teratological studies on methyl isocyanate in Charles Foster rats (part I). Biol. Memoirs 20:117– 123.

Sriramachari, S., and K.Jeevaratnam. 1994. Comparative toxicity of methyl isocyanate and its hydrolytic derivatives in rats. II. Pulmonary histopathology in the subacute and chronic phases. Arch. Toxicol. 69:45–51.

Stevens, M.A., S.Fitzgerald, M.G.Ménache, D.L.Costa, and J.R.Bucher. 1987. Functional evidence of persistent airway obstruction in rats following a two-hour inhalation exposure to methyl isocyanate. Environ. Health Perspect. 72:89–94.

ten Berge, W.F., A.Zwart, and L.M.Appleman. 1986. Concentration-time mortality response relationship of irritant and systemically acting vapors and gases. J. Hazard. Mat. 13:301–309

Tepper, J.S., M.J.Wiester, D.L.Costa, W.P.Watkinson, and M.F.Weber. 1987. Cardiopulmonary effects in awake rats four and six months after exposure to methyl isocyanate. Environ. Health Perspect. 72:95–103.

Troup, C.M., D.E.Dodd, E.H.Fowler, and F.R.Frank. 1987. Biological effects of short-term, high-concentration exposure to methyl isocyanate. II. Blood chemistry and hematologic evaluations. Environ. Health Perspect. 72:21–28.

Union Carbide (Union Carbide Corporation). 1966. Compilation of toxicology on methyl isocyanate—TSCA informational submittal with cover letter dated 121784. EPA/OTS; Doc #88–8500718. 189 pp.

Union Carbide (Union Carbide Corporation). 1973. MIC—Sensitization characteristics with cover sheets and letter dated 012591. EPA/OTS; Doc #86– 910000666D.

Uraih, L.C., F.A.Talley, K.Mitsumori, B.N.Gupta, J.R.Bucher, and G.A. Boorman. 1987. Ultrastructural changes in the nasal mucosa of Fischer 344 rats and B6C3F1 mice following an acute exposure to methyl isocyanate. Environ. Health Perspect. 72:77–88.

Varma, D.R. 1987. Epidemiological and experimental studies on the effects of methyl isocyanate on the course of pregnancy. Environ. Health Perspect. 72:153–157.

Varma, D.R. 1989. Hydrogen cyanide and Bhopal. Lancet 2:567–568.

Varma, D.R., J.S.Ferguson, and Y.Alarie. 1988. Inhibition of methyl isocyanate toxicity in mice by starvation and dexamethasone but not by sodium thiosulfate, atropine, and ethanol. J. Toxicol. Environ. Health 24:93–101.

Varma, D.R., and I.Guest. 1993. The Bhopal accident and methyl isocyanate toxicity. J. Toxicol. Environ. Health 40:513–529.

Varma, D.R., I.Guest, S.Smith, and S.Mulay. 1990. Dissociation between maternal and fetal toxicity of methyl isocyanate in mice and rats. J. Toxicol. Environ. Health 30:1–14.

Vijayan, V.K., and K.Sankaran. 1996. Relationship between lung inflammation, changes in lung function and severity of exposure in victims of the Bhopal tragedy. Eur. Respir. J. 9:1977–1982.

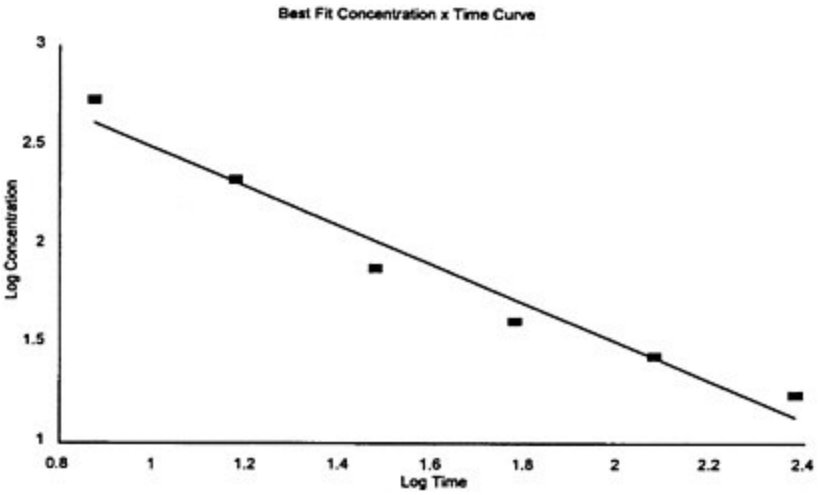
Vijayaraghavan, R., and M.P.Kaushik. 1987. Acute toxicity of methyl isocyanate and ineffectiveness of sodium thiosulphate in preventing its toxicity. Ind. J. Exp. Biol. 25:531–534.

Weill, H. 1988. Disaster at Bhopal: The accident, early findings and respiratory health outlook in those injured. Physiology 23:587–590.

Appendixes

APPENDIX A

Time-Scaling Calculations



Data: LC50 Values in the Rat (Mellon Institute 1970)

Time (min)	Concentration (ppm)	Log Time	Log Concentration
7.5	541	0.8751	2.7332
15	216	1.1761	2.3345
30	76.6	1.4771	1.8842
60	41.3	1.7782	1.6160
120	27.4	2.0792	1.4378
240	17.5	2.3802	1.2430

Regression Output:	
Intercept	3.4828
Slope	−0.9880
R Squared	0.9642
Correlation	−0.9818
Observations	6
n=1.01	
k=3351.62	

APPENDIX B

Derivation of AEGL Values

Derivation of AEGL-1

An AEGL-1 was not recommended because the irritation threshold for MIC may be above the level of concern for systemic effects such as spontaneous abortion and fetal or infant death. It is not known at what concentration the risk for these systemic effects becomes a concern. The concentrations causing irritation in humans after several minutes (1–4 ppm) are similar to, or higher than, the concentrations resulting in embryo and fetal lethality in well conducted animal studies. Therefore, it should be noted that exposures to MIC at concentrations below those used to calculate AEGL-1 might be associated with systemic toxicity. The absence of an AEGL-1 does not imply that exposure below the AEGL-2 is without adverse effects.

Derivation of AEGL-2

Key Studies:	Varma (1987) and Tepper et al. (1987)
Toxicity end point:	Decreased fetal body weights in offspring from mice exposed at 2 ppm for 3 h on GD 8 and cardiac arrhythmias in rats exposed at 3 ppm for 2 h; the concentrations were reduced by a factor of 3 to estimate a threshold for effects.
Scaling:	$C^1 \times t = k$ (based on regression analysis of LC50 values in the rat).
Uncertainty factors:	Total uncertainty factor: 30 Interspecies: 3. A factor of 3 was applied for interspecies variation because similar results have been obtained in both rats and mice. Intraspecies: 10. A factor of 10 was applied for intra

	species variation because the mechanism of action for developmental toxicity is unknown.
Calculations:	(C^1/uncertainty factors)×t=k
	([0.67 ppm]/30)^1×3 h=0.067 ppm·h; or ([1 ppm]/30)^1×2 h=0.067 ppm·h
10-min AEGL-2:	([0.067 ppm·h]/0.167 h)^1=0.40 ppm
30-min AEGL-2:	([0.067 ppm·h]/0.5 h)^1=0.13 ppm
1-h AEGL-2:	([0.067 ppm·h]/1 h)^1=0.067 ppm
4-h AEGL-2:	([0.067 ppm·h]/4 h)^1=0.017 ppm
8-h AEGL-2:	([0.067 ppm·h]/8 h)^1=0.008 ppm

Derivation of AEGL-3

Key Study:	Schwetz et al. (1987)
Toxicity end point:	Experimentally derived NOEL for pup mortality during lactation in mice exposed at 1 ppm for 6 h on gestation days 14–17
Scaling:	C^1×t=k (based on regression analysis of LC_{50} values in the rat).
Uncertainty factors:	Total uncertainty factor: 30 Interspecies: 3. A factor of 3 was applied for interspecies variation because similar results have been obtained in both rats and mice. Intraspecies: 10. A factor of 10 was applied for intraspecies variation because the mechanism of action for developmental toxicity is unknown.

Calculations:	(C^1/uncertainty factors)×t=k
	([1 ppm]/30)^1×6 h=0.2 ppm·h
10-min AEGL-2:	([0.2 ppm·h]/0.167 h)^1=1.2 ppm
30-min AEGL-2:	([0.2 ppm·h]/0.5 h)^1=0.40 ppm
1-h AEGL-2:	([0.2 ppm·h]/1 h)^1=0.20 ppm
4-h AEGL-2:	([0.2 ppm·h]/4 h)^1=0.05 ppm
8-h AEGL-2:	([0.2 ppm·h]/8 h)^1=0.025 ppm

APPENDIX C

DERIVATION SUMMARY FOR ACUTE EXPOSURE GUIDELINE LEVELS FOR METHYL ISOCYANATE (CAS No. 624–83–9)

AEGL-1[a]				
10 min	30 min	1 h	4 h	8 h
NR	NR	NR	NR	NR

[a]Exposure to MIC at concentrations below those used to calculate AEGL-1 may be associated with systemic toxicity

Abbreviation: NR, not recommended.

AEGL-2				
10 min	30 min	1 h	4 h	8 h
0.40 ppm	0.13 ppm	0.067 ppm	0.017 ppm	0.008 ppm

Key references: (1) Varma, D.R. 1987. Epidemiological and experimental studies on the effects of methyl isocyanate on the course of pregnancy. Environ. Health Perspect. 72:153–157.
(2) Tepper, J.S., M.J.Wiester, D.L.Costa, W.P.Watkinson, and M.F.Weber. 1987. Cardiopulmonary effects in awake rats four and six months after exposure to methyl isocyanate. Environ. Health Perspect. 72:95–103.

Test species/strain/number: Female mouse/Swiss-Webster/11–24; male rat/F344/ number not stated.

Exposure route/concentrations/durations: Inhalation at 2, 6, 9, or 15 ppm for 3 h on GD 8 (2 ppm was determinant for AEGL-2). Inhalation at 3, 10, or 30 ppm for 2 h (3 ppm was determinant for AEGL-2).

Effects: 2, 6, 9, and 15 ppm, reduced fetal body weights; 9 and 15 ppm, increases in complete litter resorption and maternal mortality; 30 ppm, mortality of all animals; 10 ppm, increased minute ventilation during CO_2 challenge and increased wet and dry lung weights 4 mo after exposure; 3 and 10 ppm, increase in cardiac arrhythmias 4 mo after exposure.

End point/concentration/rationale: Reduced fetal body weight and increased cardiac arrhythmias; experimental concentration reduced by a factor of 3 to estimate a threshold for effects.

Uncertainty factors/rationale:
Total uncertainty factor: 30
Interspecies: 3—developmental toxicity did not vary greatly among species.
Intraspecies: 10—the mechanism of developmental toxicity is unknown.
Modifying factor: None
Animal to human dosimetric adjustment: Insufficient data
Time scaling: $C^1 \times t = k$
Data quality and support for the AEGL values: AEGL-2 values for MIC were based on two well conducted animal studies and are supported by human data.

AEGL-3

10 min	30 min	1 h	4 h	8 h
1.2 ppm	0.40 ppm	0.20 ppm	0.05 ppm	0.025 ppm

Key reference: Schwetz, B.A., Adkins, B., Jr., Harris, M., Moorman, M., and Sloane, R. 1987. Methyl isocyanate: reproductive and developmental toxicology studies in Swiss mice. Environ. Health Perspect. 72:149–152.

Test species/strain/number: Mouse/Swiss (CD-1)/39–44

Exposure route/concentrations/durations: Inhalation at 1 or 3 ppm for 6 h/d on gestation days 14–17 (1 ppm was determinant for AEGL-3).

Effects: 3 ppm, decreased pup survival during lactation day 0–4; 1 ppm, no effects on pup survival.

End point/concentration/rationale: No effect level for pup mortality

Uncertainty factors/rationale:

Total uncertainty factor: 30

Interspecies: 3—developmental toxicity did not vary greatly among species.

Intraspecies: 10—the mechanism of developmental toxicity is unknown.

Modifying factor: none

Animal to human dosimetric adjustment: Insufficient data

Time Scaling: $C^1 \times t = k$

Data quality and support for the AEGL values: AEGL-3 values for MIC were based on a well conducted animal study and supported by other animal data.

APPENDIX D

CATEGORY PLOT FOR METHYL ISOCYANATE

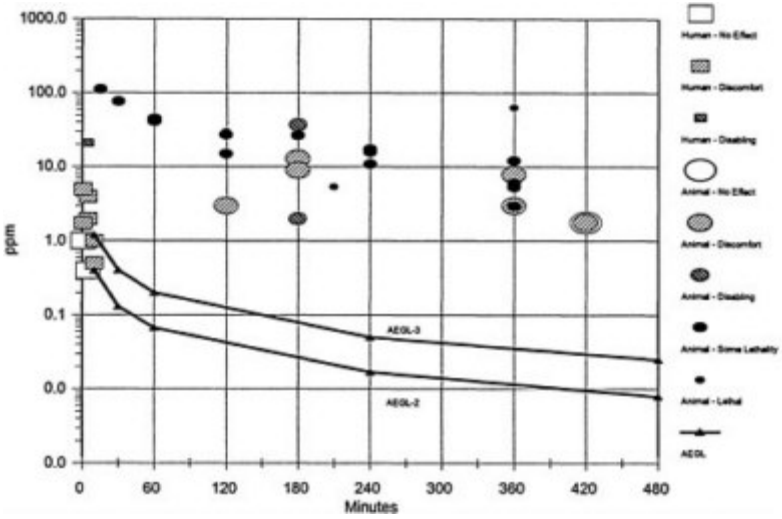


FIGURE D-1 Category plot of human and animal data compared to AEGL values.

4

Diborane[1]

SUMMARY

Diborane (CAS Registry No. 19287–45–7) is a highly unstable gas, and it is combustible upon exposure to moist air or high heat. The presence of some contaminants may lower the ignition temperature to at or below room temperature. Because of its strong reducing character, it has many industrial uses; it can be used as a rubber vulcanizer, as a catalyst for olefin polymerization, as an intermediate in the production of other boron hy

[1]This document was prepared by the AEGL Development Team comprising Claudia Troxel (Oak Ridge National Laboratory) and members of the National Advisory Committee (NAC) on Acute Exposure Guideline Levels for Hazardous Substances including James Holler (Chemical Manager) and Robert Benson and George Rodgers (Chemical Reviewers). The NAC reviewed and revised the document and AEGLs as deemed necessary. Both the document and the AEGL values were then reviewed by the National Research Council (NRC) Subcommittee on Acute Exposure Guideline Levels. The NRC subcommittee concludes that the AEGLs developed in this document are scientifically valid conclusions based on the data reviewed by the NRC and are consistent with the NRC guidelines reports (NRC 1993, 2001).

drides, and as a doping gas in the semiconductor industry. Diborane was also investigated in the 1950s as a potential rocket fuel.

Data on acute exposures of humans to diborane were limited to case reports of accidental work-related exposures. Signs and symptoms of exposure included chest tightness, shortness of breath and dyspnea, wheezing, nonproductive cough, and precordial pain. Workers exposed to diborane generally experienced a complete recovery within a short period following cessation of exposure. No quantitative information was given regarding the exposure terms of these individuals, and the data were therefore unsuitable for derivation of AEGLs. No reports of human fatalities after diborane exposure were found in the literature. Reported odor thresholds range from 1.8 parts per million (ppm) to 3.6 ppm.

Data on lethal and nonlethal consequences of diborane exposure were available for several animal species, including dogs, rats, mice, hamsters, rabbits, and guinea pigs. Fifteen-minute LC_{50} values in rats ranged from 159 ppm to 182 ppm, and 4-hour (h) LC_{50} values ranged from 40 ppm to 80 ppm in rats and 29 ppm to 31.5 ppm in mice. Animals exposed to lethal and nonlethal concentrations developed pulmonary hemorrhage, congestion, and edema, and death was related to these severe pulmonary changes. Recent studies in rats and mice have also uncovered the development of multifocal and/or diffuse inflammatory epithelial degeneration in the bronchioles following exposure to diborane. These pulmonary changes produced by exposure to nonlethal concentrations were completely reversible in rats by 2 weeks (wk) after an acute exposure and were being repaired in the mouse by 2 wk postexposure. The signs of toxicity and repair of pulmonary lesions following acute exposure to nonlethal concentrations in animals were similar to the human case reports. It is likely that the mechanism of toxicity is due to direct interaction of diborane with cellular components, especially because diborane is such a potent reducer. There appears to be a similar mechanism of toxicity among species, because the cause of death from diborane exposure has always been from pulmonary damage, including edema, hemorrhage, and congestion. Mice appeared to be the more sensitive species, and the mice data were therefore used for the derivations of AEGLs.

An AEGL-1 value was not recommended because the AEGL-2 value is below the odor threshold of diborane and no other data pertaining to end points relevant to AEGL-1 definition were available. Absence of an AEGL-1 does not imply that exposure below the AEGL-2 is without adverse effects.

The AEGL-2 values were based on reversible histological changes in the lungs in male ICR mice following a 2-h acute inhalation exposure to diborane at 5 ppm. No effects were observed in mice exposed at 5 ppm for 1 h, and exposure at 5 ppm for 2 h resulted in 4/10 mice developing multifocal and/or diffuse inflammatory epithelial degeneration in the bronchioles (Nomiyama et al. 1995). Studies have demonstrated that these lesions are reversible. There were no other treatment-related changes, such as changes in behavior, appearance, body or organ weight, or hematological or clinical chemistry indices. A total uncertainty factor (UF) of 10 was applied to the AEGL-2 value. An interspecies UF of 3 was applied because the most sensitive species, the mouse, was used and the end point of toxicity, reversible histological changes in the lungs, was the most sensitive end point. Further support for the UF of 3 is that signs of toxicity and repair of pulmonary lesions following acute exposure to nonlethal concentrations of diborane in animals were consistent with the human response reported by case reports. There appears to be a similar mechanism of toxicity among species because the cause of death from diborane exposure is due to acute pulmonary damage, including edema, hemorrhage, and congestion. An intraspecies UF of 3 was applied because using the default UF of 10 generates AEGL values that are inconsistent with existing empirical data. For example, the derived 1-h AEGL-2 value is 1.0 ppm with a total UF of 10. Mice exposed at 1 ppm for up to 8 h exhibited no effects of diborane exposure (Nomiyama et al. 1995). In addition, mice exposed at 0.7 ppm for 6 h/day (d), 5 d/wk for up to 4 wk developed only slight pulmonary infiltration of polymorphous neutrophils (Nomiyama et al. 1995) and rats exposed at 0.96 ppm for 6 h/d, 5 d/wk for 8 wk developed changes in bronchoalveolar lavage fluid that were not accompanied by histopathological changes (Nomiyama et al. 1996). The use of a higher UF would result in AEGL values that would be below concentrations causing effects in any species for an end point that is supposed to be disabling or cause irreversible effects in a human population.

The AEGL-3 values were based on the estimate of a 4-h LC_{01} of 9.2 ppm obtained by log-probit analysis of data from a 4-h LC_{50} study in male ICR mice (Uemura et al. 1995). A total UF of 10 was applied to the AEGL-3 value. An interspecies UF of 3 was applied because there did not appear to be much variation between species in sensitivity to lethal concentrations of diborane. The 4-h LC_{50} values determined by different authors for mice and rats were within a factor of 2.8 (4-h LC_{50} values ranged from

29 ppm to 31.5 ppm in mice and from 40 ppm to 80 ppm in rats). The lung was the target organ in all species tested, and the biological response remained the same, becoming more severe with increasing concentrations until death occurred from anoxia as a consequence of severe pulmonary changes. An intraspecies UF of 3 was applied because using the default UF of 10 generates AEGL values that are inconsistent with existing empirical data. For example, the derived 1-h AEGL-3 value is 3.7 ppm with a total UF of 10. Mice exposed at 5 ppm for up to 4 h developed only inflammatory epithelial degeneration in the bronchioles, with exposure for 8 h resulting in increased lung weights (Nomiyama et al. 1995). Mice exposed at 15 ppm for 4 h developed pulmonary changes, including edema, congestion, and inflammatory epithelial degeneration, that were generally resolved or in the process of being resolved within 14 d postexposure (Uemura 1996). The use of a higher UF would result in AEGL values that would be below concentrations causing effects in any species for an end point which is supposed to be life-threatening in a human population.

The derived AEGL values were scaled to 10-minute (min), 30-min, 1 -h, 4-h, and 8-h exposures using $C^n \times t = k$. To calculate n for diborane, a regression plot of the EC_{50} values was derived from the studies by Nomiyama et al. (1995) and Uemura et al. (1995) investigating 1-, 2-, and 4-h exposures at 1, 5, or 15 ppm, with multifocal and/or diffuse inflammatory epithelial degeneration in the bronchioles as the end point of toxicity. Although *n* values have generally been derived using lethality data, it was considered appropriate in this case to use the nonlethal pulmonary changes. Toxicity studies demonstrated that the lung remained the target organ at all concentrations of exposure, and the biological response remained the same, becoming more severe with increasing concentration until death occurred from anoxia as a consequence of severe pulmonary changes. From the regression analysis, the derived value of $n=1$ was used in the temporal scaling of all the AEGL values ($C^1 \times t = k$; Haber's law). The 10-min AEGL-3 value was set equal to the 30-min value of 7.3 ppm because the NAC considers it inappropriate to extrapolate from the exposure duration of 4 h to 10 min. Although it is considered appropriate to extrapolate from a 2-h exposure to a 10-min exposure duration in the AEGL-2 derivation, the 10-min value of 6.0 ppm would approach that of the 10-min AEGL-3 value of 7.3 ppm. Therefore, the 10-min AEGL-2 value was set equal to the 30-min value. The AEGL values are listed in the table below.

TABLE 4–1 Summary of AEGL Values for Diborane

Classification	10 min	30 min	1 h	4 h	8 h	End Point (Reference)
AEGL-1 (Nondisabling)	NR[a]	NR	NR	NR	NR	Not recommended because the AEGL-2 value is below the odor threshold, and no other data pertaining to end points relevant to the AEGL-1 definition were available
AEGL-2 (Disabling)	2.0 ppm (2.2 mg/m^3)	2.0 ppm (2.2 mg/m^3)	1.0 ppm (1.1 mg/m^3)	0.25 ppm (0.28 mg/m^3)	0.13 ppm (0.14 mg/m^3)	LOAEL for pulmonary changes in male ICR mice; 5 ppm for 2 h (Nomiyama et al. 1995)
AEGL-3 (Lethality)	7.3 ppm (8.0 mg/m^3)	7.3 ppm (8.0 mg/m^3)	3.7 ppm (4.1 mg/m^3)	0.92 ppm (1.0 mg/m^3)	0.46 ppm (0.51 mg/m^3)	4-h LC_{01} of 9.2 ppm estimated from a 4-h LC_{50} in male ICR mice (Uemura et al. 1995)

[a]Absence of an AEGL-1 does not imply that exposure below the AEGL-2 is without adverse effects.
Abbreviation: NR, not recommended.

1. INTRODUCTION

Although the boron hydrides were first described in 1879, their possible uses were not investigated until the military became interested in their potential for use as rocket fuels in the 1950s (Rozendaal 1951; Stumpe 1960). The three most studied boron hydrides were pentaborane, a liquid, decaborane, a solid, and diborane, a gas. Diborane is highly unstable and can spontaneously combust at temperatures of 40–50 °C. The presence of contaminants may lower the ignition temperature to at or below room temperature (Budavari et al. 1996). It rapidly hydrolyzes in water to produce boric acid, hydrogen, and heat. Because of its strong reducing character, diborane has many industrial uses; it is used as a rubber vulcanizer, a catalyst for olefin polymerization, an intermediate in preparation of other boron hydrides, and a doping gas in the semiconductor industry (Budavari et al. 1996). "Certain base adducts of borane, BH_3, such as $(C_2H_5)_3N.BH_3$ [1722– 26–5], $(CH_3)_2S.BH_3$ [13292–87–0], tetrahydrofuranborane [14044–65–6], and $C_4H_8O.BH_3$ are more easily and safely handled than B_2H_6 and are commercially available. They find wide use as reducing agents and in hydroboration reactions" (Rudolph 1978). Currently, diborane is one of the most used speciality gases in the semiconductor industry in Japan (655 kg consumed in 1993), and its increasing usage has prompted more refined toxicity studies than were previously available in the literature (Nomiyama et al. 1996). Information on diborane production and use data in the United States is limited. Two companies in the United States are listed as producing diborane: one having the capacity to produce 45 metric tons per year, and the other producing diborane on demand. Dopants in general, including boron trifluoride, diborane, arsine, and phosphine, were predicted to have a 9% average annual growth rate between 1994 and 1999 (Chemical Economics Handbook 1996). The physicochemical data of diborane are presented in Table 4–2.

The odor of diborane is described as repulsive and sickly sweet (Budavari et al. 1996). The median detectable odor concentration of diborane was determined to be 2–4 mg/m^3 (1.8–3.6 ppm) (Krackow 1953) and 2.5 ppm (Amoore and Hautala 1983), which is above the occupational exposure limits set for this compound (ACGIH 1991, 1996). Toxicity data in humans were limited to case reports. Studies addressing lethal, nonlethal, and reproductive toxicity of diborane in experimental animals were available.

TABLE 4–2 Chemical And Physical Data

Parameter	Value	Reference
Synonyms	Boroethane, diboron hexahydride, boron hydride	Budavari et al. 1996; ACGIH 1991
Molecular formula	B_2H_6	Budavari et al. 1996
Molecular weight	27.67	Budavari et al. 1996
CAS Registry Number	19287–45–7	ACGIH 1991
Physical state	Gas	Budavari et al. 1996
Color	Colorless	Budavari et al. 1996
Solubility	Hydrolyzes in water	Budavari et al. 1996
Vapor pressure	>1 atm at 20 °C 27,460 mm Hg (15 °C)	ACGIH 1991; Lockheed Martin Energy Systems, Inc. 1988
Specific gravity (water=1)	0.210 (15 °C)	Budavari et al. 1996
Density (air=1)	0.965	Braker and Mossman 1980
Melting point	−165 °C	Budavari et al. 1996
Boiling point	−92 5 °C	Budavari et al. 1996
Flammability limits	Spontaneous ignition in air at 40– 50 °C	Budavari et al. 1996
Conversion factors	1 ppm=1.1 mg/m^3 1 mg/m^3 =0.91 ppm	ACGIH 1996

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

LC_{50} values in humans have been reported to be 159 ppm for 15 min, and 30–90 mg/m^3 (27–82 ppm) for 4 h (Braker and Mossman 1980;

Lockheed Martin Energy Systems, Inc. 1988). However, no references were cited and no information was given regarding the derivation of these values. These values are therefore inappropriate for use in derivations of AEGLs.

2.2. Nonlethal Toxicity

2.2.1. Case Reports

There are no studies in humans reporting the effects following exposures to known concentrations of diborane. The case reports in the literature concerning accidental workplace exposures provide some characterization of signs and symptoms associated with diborane poisoning. It is doubtful that workplace exposures were limited to diborane alone, but most likely included exposures to other chemicals such as the similar boron hydrides decaborane and pentaborane.

In 1957, Rozendaal summarized case reports of workers exposed to boron hydrides. One worker developed fatigue, shortness of breath, chills, and fever a few hours after diborane exposure. He was later diagnosed with "pneumonia" and treated with penicillin and made a complete recovery in 3 d. In this paper, Rozendaal compared the symptoms that developed following diborane exposure with those of "metal fume fever."

Lowe and Freeman (1957) conducted a survey of dispensary records and laboratory data from 83 people who were potentially exposed to boron hydrides during a 3 y period. They noted that 2 out of 38 people exposed to diborane were hospitalized. Commonly reported symptoms of exposure included tightness, heaviness, and burning sensations of the chest, shortness of breath, a nonproductive cough, and precordial pain. Chest X-rays from the two hospitalized patients showed nonspecific infiltration, which cleared up in 1–2 d. Chronic exposures to low levels of diborane were associated with central nervous system-type symptoms, including lightheadedness, dizziness, vertigo, chills, and fever. Muscular weakness and fatigue were often noted but were generally gone by the next day. Tremors, which seldom occurred, were localized and of short duration.

Rousch (1959) described similar symptoms in workers exposed to diborane and commented that if exposed men were asked to describe their symptoms most referred to cough, chest tightness, and headache, adding that only a "few whiffs" of the gas were needed to develop the symptoms.

The symptoms tended to develop within a few minutes and generally lasted a few hours.

Cordasco et al. (1962) described the effects of boron hydride exposures recorded between 1956 and 1960. Of the 26 cases of acute diborane exposures, 18 exhibited respiratory problems, including chest tightness and pain, dyspnea, nonproductive cough, and wheezing, generally lasting from 3 to 5 d. Ten percent of the cases experienced nausea, anorexia, and hyper-salivation. There were 33 reported cases of subacute exposures, with 8 cases of respiratory involvement. Symptoms associated with exposure to low concentrations for longer periods included chest tightness, nonproductive cough, lightheadedness, headache, fatigue, and drowsiness. Inspiratory and expiratory rhonchi were the most prominent clinical findings during chest examinations of patients exposed both on an acute and subacute basis. Cordasco also recounted two case reports of acute exposures to diborane. One exposed worker developed breathing difficulty, severe tightness in the upper chest, weakness, and slight twitching of the hands, all of which continued for 2 h. Dyspnea and cough continued over the next 3 d, and rales were heard in both lungs. A chest X-ray showed infiltration in both lungs. Five days after exposure, the patient's cough and dyspnea were gone, and his lungs were clear. Thirteen days later, the worker was again exposed to diborane, and he experienced severe shortness of breath and diffuse chest tightness. Examination indicated medium dry rales in the posterior bases of the lungs, and a chest X-ray showed "pneumonitis." The patient was treated with penicillin and chloramphenicol, and he was asymptomatic 7 d later. A chest X-ray 3 wk after exposure showed a disappearance of the lesions. The second patient exposed to diborane immediately developed shortness of breath, vertigo, and dry cough. He was given oxygen for 20 min, after which he felt fine. He was again exposed to diborane 6 d later and developed a dry cough. He had moist rales at both bases of the lungs 7 d later, and an X-ray taken 9 d after the second exposure revealed pneumonitis in both bases. The patient received treatment with penicillin and isoproterenol and returned to normal shortly thereafter.

2.2.2. Epidemiology Studies

Epidemiologic studies regarding human exposure to diborane were not found in the available literature.

2.2.3. Other

Reported odor thresholds for diborane are 2–4 mg/m^3 (1.8–3.6 ppm) (Krackow 1953) and 2.5 ppm (Amoore and Hautala 1983).

2.3. Developmental and Reproductive Effects

No human developmental and reproductive toxicity data concerning diborane were found in the available literature.

2.4. Genotoxicity

No human genotoxicity data on diborane were found in the available literature.

2.5. Carcinogenicity

No data were found in the available literature regarding the carcinogenic potential of diborane.

2.6. Summary

While there were several case reports describing the effects of diborane exposure in humans, exposure durations and concentrations were missing, and it was doubtful that workplace exposures were limited to just diborane. Commonly reported signs and symptoms associated with acute diborane exposure included chest tightness, nonproductive cough, dyspnea, precordial pain, fatigue, and wheezing. The symptoms developed shortly after exposure, and generally disappeared within a week. Three patients showed signs of apparent “pneumonitis” or “pneumonia” and experienced a complete recovery. Repeated and chronic exposures produced signs and symptoms such as headache, lightheadedness, fatigue, dizziness, chest tightness, and cough. No carcinogenicity, reproductive, or developmental toxicity data in humans were available.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Dogs

A dog anesthetized by intravenous administration of pentobarbital sodium was exposed to diborane at 350 ppm for 15 min by intratracheal cannulation (Kunkel et al. 1956). The concentration of diborane was calculated from the measured rates of air and diborane flow into a gas chamber, and the mixture was then delivered from the chamber to the animal by a polyethylene tube. The exposed dog's blood pressure began to drop and its respiration and thoracic movements were increased within 5 min of gassing. The animal died 4 min after exposure ceased, by which time edema fluid was noted to be flowing from the tracheal cannula. A terminal ECG showed sinus bradycardia and increased T-wave voltage. Signs of pulmonary congestion, hemorrhage, and edema were found during necropsy. In addition, the liver was congested and casts were found in the renal tubules.

Using the same method of exposure, three more anesthetized dogs were exposed at 40–125 ppm for 2–2.5 h (Kunkel et al. 1956). Exposure to diborane increased intestinal peristalsis in all dogs and produced hyperactivity of the EEG in two of the three dogs. One of these dog's EEG returned to normal, while the other dog exhibited depressed cortical activity followed by bradycardia and finally ventricular fibrillation leading to death. Pulmonary edema was found in the dog during necropsy. A second dog had died by the end of the experiment, showing gross evidence of pulmonary edema. The dogs used by Kunkel et al. were of mixed breed and gender.

Comstock et al. (1954) exposed male beagle dogs to diborane at 6 or 0.8–1.7 mg/m^3 (5 or 0.7–1.5 ppm) in a gassing chamber for 6 h/d, 5 d/wk, for up to 6 months (mo). By the twenty-fifth exposure, there was 100% mortality (2/2) in the dogs exposed at 6 mg/m^3 (5 ppm). The dogs developed respiratory distress as soon as the first exposure, and exhibited signs of respiratory infection by the ninth exposure. Pathological examination of one of the dogs revealed acute and chronic nasopharyngitis, acute tracheitis, chronic bronchitis and bronchopneumonia, and liver and kidney congestion. At the lower concentrations, death occurred in one of two dogs after 130 exposures. The dog that died exhibited hyperpnea and anorexia,

and necropsy of the other dog after 6 mo of exposure found no changes attributable to treatment.

3.1.2. Rats

Krackow (1953) reported the results of a 4-h LC_{50} study conducted in rats. Rats were exposed to diborane concentrations ranging from 45 ppm to 100 ppm for 4 h. The 4-h LC_{50} was determined to be approximately 50 ppm. Affected rats showed pulmonary edema and hemorrhage upon necropsy. The author did not give many details about how the study was conducted, such as the number and gender of the animals used, the methods of exposure, if the concentrations were measured or nominal, and the study duration.

Krackow (1953) also reported the results of a 15-min LC_{50} study in rats. The 15-min LC_{50} was approximately 175–200 mg/m^3 (159–182 ppm), with death typically occurring within 2 h. Krackow then described the overall purpose of this experiment, which was to test the effectiveness of several therapeutic drugs on decreasing mortality following diborane exposure. The description of the study matches the study design and results of a paper by Kunkel et al. (1956), and is most likely the same study. Groups of 10 albino rats were exposed at approximately 175 ppm in a gas chamber for 15 min and then observed up to 10 d following exposure. Although some therapeutic drugs produced a slight decrease in mortality rate, all animals exposed to diborane had evidence of pulmonary edema and hemorrhage upon postmortem examination. In addition, casts were noted in the renal tubules. Death typically occurred within a few hours after exposure.

Comstock et al. (1954) investigated acute and chronic diborane exposures in male albino rats. To assess acute toxicity, groups of six male rats were exposed to diborane at 52–492 mg/m^3 (47–446 ppm) in a glass jar for periods of time ranging from 60 to 240 min. No controls were used. The mortalities resulting from acute exposures are as follows: 2/6 rats exposed at 47 ppm for 240 min; 46/54 rats exposed at 60–140 ppm for 240 min; 22/24 rats exposed at 158–446 ppm for 60 min; 3/6 exposed at 159 ppm for 120 min; and 6/6 exposed at 228 ppm for 120 min. Exposed animals exhibited respiratory distress, and the animals exposed to the two highest concentrations of diborane (287 and 446 ppm) died within 2 h of exposure, experiencing difficulty in breathing and gasping before death. The remaining mortalities generally occurred within 5 d after exposure. Necropsy

revealed pulmonary congestion and edema with focal areas of hemorrhage. To assess chronic toxicity, 18 rats were exposed at 6 mg/m^3 (5 ppm), and 20 rats were exposed at 0.8–1.7 mg/m^3 (1–2 ppm) for 6 h/day, 5 d/wk, for up to 6 mo. Groups of 10 rats were used as controls. At the highest concentration (5 ppm), 17/18 exposed rats died. At the lower concentrations, death occurred in 5/10 rats exposed 21 times and in 5/10 rats exposed 60 times. Comstock reports that the deaths of the rats were distributed between the seventh and one hundred and thirteenth exposure. The only sign of toxicity in rats was mild rhinitis, and limited pathological examinations of the animals did not indicate any abnormalities. The Comstock et al. study has been criticized for its lack of control data (for the acute study), limited pathological data (especially the lack of changes in both rats and guinea pigs), and lack of evidence of the cause of mortality in the rats (EPA 1988).

Jacobson and Lawson (1962) investigated the effects of age and strain on the 4-h LC_{50} value in male rats following exposure to diborane in a gassing chamber. The two strains of rats investigated were a derived Wistar strain (CRDL) and rats from Edgewood Breeding Farms (EBF). The authors noted that the rats were affected by chronic murine pneumonia, which was a common affliction in laboratory rodents at that time. The first experiment determined that the 4-h LC_{50} values in 2-mo-old EBF rats and 5-mo-old CRDL rats were 40 and 80 ppm, respectively. The second experiment examined 2- and 5-mo-old EBF rats and 5-mo-old CRDL rats. These animals were exposed at 53 ppm for 4 h, and the LC_{50} values were derived using a slope function calculated from previous experiments. Although the authors felt this slope function was appropriate because of the identical slope functions observed in previous experiments, it is inappropriate to calculate an LC_{50} from a single exposure because there is too much uncertainty. The LC_{50} values were estimated to be 42, 65, and 74 ppm for 2- and 5-mo-old EBF rats and 5-mo-old CRDL rats, respectively. The LC_{50} values for the 2-mo-old EBF and 5-mo-old CRDL rats were comparable to those determined in the previous experiment. The authors concluded there was no significant difference in the sensitivity between the two strains of rats investigated, but the age of the animals did appear to have a substantial effect on susceptibility. The authors were not sure if this difference was an effect of age or weight. The authors also recorded signs of toxicity in exposed rats, which included labored breathing and froth from the nose. Necropsy showed edematous lungs in rats which were killed the day of exposure, but lungs from rats killed later appeared normal, suggesting that

the lesions were repaired. When edema was present, it was observed both macroscopically and microscopically, and was sometimes confined to peribronchial and perivascular lymphatic spaces.

3.1.3. Mice

Female mice from Carworth Farms (CF), approximately 2.5 to 3 mo old, were exposed to various concentrations of diborane in a dynamic exposure chamber for 4 h (actual exposure concentrations not provided) (Jacobson and Lawson 1962). They were observed for 14 d postexposure, and the total mortality rate over this period was used to calculate the 4-h LC_{50} of 29 ppm. The authors recorded signs of toxicity in the exposed mice, which included labored breathing and froth from the nose.

Groups of 10 4-wk-old male ICR mice were exposed to air containing measured concentrations of diborane at 0, 11.3, 22.1, 35.1, 37.7, or 44.8 ppm in a dynamic exposure chamber for 4 h (Uemura et al. 1995). A toxic gas monitor was used to measure the diborane chamber concentrations at 1-min intervals. The animals were observed for 2 wk postexposure. There was a concentration-response relationship between exposure levels and body-weight suppression (absolute values not provided). All treatment groups had a severe decrease in body weight for 3 d following exposure, returning to normal 4 or 5 d after exposure. The only exception was the high-concentration group, which rebounded 8 d after exposure. Mortality typically occurred within 24 h of exposure. The mortality rate in the study was 0/10, 3/10, 3/10, 9/10, and 9/10 at each concentration (11.3, 22.1, 35.1, 37.7, or 44.8 ppm, respectively), and the 4-h LC_{50} was calculated to be 31.5 ppm.

In the same paper, Uemura et al. (1995) investigated the effects of acute exposure in male ICR mice following exposure to diborane at 15 ppm for 1, 2, 4, or 8 h (10 mice per group). The mice were observed for 3 d and then sacrificed. One mouse from the 8-h exposure group died before the end of the study period. The histopathological examination showed findings consistent with pulmonary irritation, including mucous exudate, degeneration and necrosis of the epithelial lining, and inflammatory cellular infiltration into the nasal cavity.

3.1.4. Rabbits

Kunkel et al. (1956) exposed anesthetized rabbits (mixed breed and gender) by head exposure to an unknown concentration of diborane gas until the animals died. These rabbits were anesthetized by intravenous administration of approximately 30 mg pentobarbital sodium per kilogram body weight. The rabbits exhibited voluntary apnea upon first contact with the gas, eventually returning to normal respiration. There was a sharp decrease in blood pressure and bradycardia before death, leading to ventricular fibrillation or cessation of ventricular activity. Death was caused by pulmonary edema.

3.1.5. Hamsters

Separate groups of male and female golden hamsters were exposed to diborane at 50, 75, 100, 150, 200, 300, 400, 500, 600, 800, or 1,000 ppm in a dynamic exposure chamber, and control animals were exposed to filtered air (Stumpe 1960). The diborane was first mixed with nitrogen and then diluted with compressed air and fed into the exposure chamber. Diborane concentration was calculated using the measured flow rates of diborane and the diluents. The exposures were terminated when all of the animals in the respective group had died. The lungs were the target organ of diborane toxicity, and death was from pulmonary edema. The mean exposure time to death decreased with increasing diborane concentrations from 50 ppm to 600 ppm (from 497 min to 33 min, respectively). From 600 ppm to 1,000 ppm, the mean exposure time to death did not change, suggesting that the minimum time required for irreversible pulmonary changes leading to death was approximately 30 min. There was an earlier onset of toxicity with increasing diborane concentrations. At the beginning of the exposures, the animals huddled together and activity subsided. Soon after, the rate and depth of respiration in the animals were observed to increase, and the animals became restless and pawed at their faces. As the exposures progressed, the animals became increasingly active and would at times fall onto their sides or backs, but were able to right themselves until near death. When near death, the animals took deep, prolonged, and gasping breaths and had periods of apnea. A pinkish froth coming from the nares and mouths was noted in animals in the 500- to 1,000-ppm treatment groups.

Necropsy showed that changes were confined to the lungs. The color of the lungs was a bright red in exposed groups, progressing to a reddish brown in the higher exposed groups. No gross liver or kidney changes were noted. Microscopic examination of the lungs revealed capillary dilation and vascular congestion, edema, focal areas of atelectasis and peripheral emphysema, and sloughing of the mucosal epithelium of the bronchioles and bronchi with degeneration of mucosal cells. Pulmonary changes were more severe in the 500- to 1,000-ppm treatment groups. Vascular congestion, particularly of the glomerular capillaries, was found in the kidneys. The gender or weight of the animals did not appear to influence the sensitivity of the animal to diborane poisoning.

3.2. Nonlethal Toxicity

3.2.1. Dogs

Kunkel et al. (1956) exposed dogs (mixed breed and gender) anesthetized with pentobarbital sodium to various concentrations of diborane by intratracheal cannulation. The concentration of diborane was calculated from the measured rates of air and diborane flow into a gas chamber, and the mixture was then delivered from the chamber to the animal by a polyethylene tube. In one experiment, three dogs were exposed to diborane at 6–14 ppm for 45 min to 4 h. The results of testing during this exposure period showed normal electrocardiograms, no effects on blood pressure, and only slight increases in respiration; however, postmortem examination showed severe pulmonary hemorrhages and slight pulmonary edema. In another experiment, three dogs were exposed at 53–63 ppm for 15 min. The animals exhibited increased rate and depth of respiration. The dog exposed at 63 ppm showed a drop in blood pressure and bradycardia, whereas the other two dogs had normal electrocardiograms. Pulmonary congestion, hemorrhage, and some edema were found on necropsy. Other organs showed some congestion but otherwise appeared normal. Another dog was exposed at 5 ppm diborane for 4 h on one day and 3 h the next day. Near the end of the second exposure, the rate and depth of respiration increased and blood pressure decreased. It was noted that the dog appeared to be in shock after the second exposure, and the authors speculated that the prolonged period of anesthesia might have been the cause. Necropsy revealed pulmonary congestion and petechial hemorrhage in the medulla of the

kidneys. Lastly, one dog exposed at 125 ppm for 30 min showed increased peristalsis of the small intestine within minutes, and the EEG activity increased after 30 min of exposure. However, all activity had returned to normal within 1 h after exposure. The dog was killed 5 h after exposure, at which time there were no signs of pulmonary edema or cyanosis.

3.2.2. Rats

Nomiyama (1995) investigated the effects of acute inhalation exposure of diborane on bronchoalveolar lavage fluid (BALF) and blood parameters in male Wistar rats. Rats were placed in a dynamic exposure chamber, and diborane concentrations were measured at 1-min intervals with a toxic gas monitor. In the first phase of the study, groups of 10 rats (8-wk-old) were exposed at 20 ppm for 4 h and control rats were exposed to filtered room air. The rats were killed immediately, 1 d, 3 d, or 14 d after exposure. Eight rats per group were used to analyze changes in BALF and blood, and two rats per group were examined for pulmonary histopathological changes. The organs of all rats were weighed and examined grossly. No differences in behavior, external appearance, or body weight were observed during the study. There were only sporadic changes in the hematology and clinical chemistry of the animals, and these changes did not indicate any time-related trends. BALF analysis revealed that the proportion of neutrophils, activities of α_1-antitrypsin and superoxide dismutase, and levels of total phospholipids were initially increased, but all returned to normal by 2 wk with the exception of α_1-antitrypsin activity, which had decreased but was still statically elevated compared with controls. Histopathological examination showed infiltration of polymorphonuclear neutrophils into the bronchus the day of exposure, becoming milder 1 and 3 d after exposure. Multifocal and/or diffuse inflammatory epithelial degeneration in the respiratory bronchioles was observed 3 d after exposure, but these lesions were not detected 2 wk after exposure.

In the second phase of this study, Nomiyama (1995) investigated similar biological end points 3 d after a 4-h exposure to diborane at 0, 0.9, or 9.2 ppm (control rats were exposed to filtered room air). Each group contained 12 male Wistar rats (13-wk-old): 10 per group were used for BALF analysis and 2 per group were used for histopathological evaluation. The organs of all animals were weighed and examined grossly. The liver-to-body weight ratio was significantly decreased in the high-concentration

group (absolute value not provided). BALF analysis showed a number of changes, including concentration-related increases in total protein, α_1-antitrypsin activity, and total phospholipids, increases in lactate dehydrogenase (LDH) and in the proportion of neutrophils and lymphocytes, and a concentration-dependent decrease in the proportion of macrophages. The activity of serum α_1-antitrypsin was increased in the 9.2-ppm exposure group. Histopathological examination revealed inflammatory epithelial degeneration in the bronchioles in the high-concentration group.

Groups of 12 14-wk-old male Wistar rats were exposed at 0.11 or 0.96 ppm (control rats were exposed to filtered room air) for 8 wk (6 h/d, 5 d/wk) in dynamic exposure chambers (Nomiyama et al. 1996). Diborane concentration was measured at 1-min intervals using a toxic gas monitor. At the conclusion of the exposures, two rats per group were examined for histopathological lesions, while the other 10 per group were assigned to BALF analysis. The organs of all rats were weighed and examined grossly. The liver-to-body weight ratio was significantly decreased in the high-concentration group (absolute value not provided). BALF analysis indicated that the proportion of neutrophils and the levels of alkaline phosphatase increased with concentration, while the percentage of macrophages decreased in the 0.96-ppm group only. The high-concentration group also had a significant increase in the total and individual number of phospholipids and lactate dehydrogenase (LDH) activity. Analysis of the serum indicated increases in α_1-antitrypsin and superoxide dismutase activities in both exposure groups. The changes measured in BALF and serum, which signified inflammation and cell damage, exhibited a concentration-dependent effect in the lungs. Despite changes in biochemical markers, however, there were no histopathological, behavioral, or external changes noted in any of the treatment groups, suggesting that the observed changes should be reversible following cessation of exposure to diborane.

Krackow (1953) summarized preliminary results of an experiment assessing chronic toxicity in rats (gender and strain not given) exposed at 1 to 4 mg/m^3 (0.9–3.6 ppm) for 6 h/d, 5 d/wk. At that time, the experiment was in its fourth month. Pathological pulmonary changes, varying from congestion to pneumonia, were observed 1 to 4 wk after exposures started. Microscopic evaluation of pulmonary tissue showed round cell infiltration of the tracheal mucosa. Other organs examined sometimes showed congestion but were otherwise normal. No mortality had occurred at that time point. The paper discussing the final results of this study was not found in the available literature. Krackow also referred to repeated dose studies in

which rats (strain and gender not given) exposed at 6 mg/m^3 (5.4 ppm) for 6 h/d, 5 d/wk, showed evidence of pulmonary damage in 2–3 wk.

3.2.3. Mice

Uemura et al. (1995) investigated acute effects of diborane on 5-wk-old male ICR mice. Ten mice per group were exposed in a dynamic exposure chamber to diborane at 15 ppm for 1, 2, 4, or 8 h or to filtered room air for 8 h. Diborane concentrations were measured at 1-min intervals with a toxic gas monitor. Actual exposure concentrations at the various time periods were 12.3, 13.1, 13.6, and 14.4 ppm, respectively. Mice were sacrificed 3 d after exposure. During the exposure periods, the mice in the treatment groups exhibited signs such as face washing movements and restlessness, and some mice in the 4- and 8-h groups had ruffled fur and systemic tremors. Upon termination of the experiment, mice from the 2-, 4-, and 8-h exposure groups had statistically decreased body weights (93%, 84%, and 86% of controls, respectively) and increased lung and trachea weights (125%, 129%, and 142% of controls, respectively). In addition, mice from the 4- and 8-h exposure groups had statistically decreased weights of the liver (83% and 79% of controls, respectively) and kidney (90% of controls). There were no hematological or clinical chemistry changes related to treatment. Histopathological examination demonstrated a time-response relationship for multifocal and/or diffuse inflammatory epithelial degeneration in the bronchioles. Clara cells with mitotic figures were frequently observed in the inflamed epithelium. The longer exposed mice had more frequent and severe cellular infiltration in the respiratory bronchioles, congestion, edema, and bleeding (see Table 4–3). The increasing severity of these lesions with time was probably due to direct contact of diborane with the epithelium. The one dead mouse from the 8-h exposure group had findings consistent with pulmonary irritation, including mucous exudate, degeneration and necrosis of the epithelial lining, and inflammatory cellular infiltration in the nasal cavity.

Groups of 10 5-wk-old male ICR mice were exposed to diborane at 15 ppm for 4 h and sacrificed immediately, 1 d, 3 d, or 2 wk after exposure, and controls were exposed to filtered room air for 4 h and sacrificed immediately or 2 wk after exposure (Uemura 1996). Exposures were conducted in a dynamic exposure chamber, and diborane concentrations were mea

sured at 1-min intervals with a toxic gas monitor. Face-washing and restlessness were observed soon after the exposure started, and ruffled fur and hypoactivity were noted in some mice in the 3-d and 14-d postexposure groups. Immediately after diborane exposure, infiltration of polymorphonuclear neutrophils at the bronchiolus and some infiltration of macrophages into the alveoli were observed. Macrophage infiltration into the alveoli became more prominent 1 d and 3 d postexposure. Inflammatory epithelial degeneration, edema, and congestion were present in the lungs of all of the mice by 1 d postexposure, becoming more severe 3 d postexposure. Lung weight increased with the severity of the pulmonary lesions (see Table 4–4). Electron microscope analysis showed deposition of fine, fibrillar materials in the alveoli and bronchiolus immediately, with evidence of phagocytosis by macrophages 3 d after exposure. The nature of the fibrillar materials was unclear; boron atoms were not detected by disperse X-ray analysis. By 2 wk postexposure, the edema and congestion had almost completely subsided; however, lung weights were still significantly increased. Peribronchiolar thickening and infiltration of inflammatory cells into the bronchiolar walls were observed where inflammatory epithelial degeneration had previously been detected. Infiltration of lymphocytes into the subepi

TABLE 4–3 Prevalence of Microscopic Lesions in Lungs of Mice Exposed at 15 ppm Diboranea

	Exposure Time				
Microscopic Finding	Control	1 h	2 h	4 h	8 h
Inflammatory epithelial degeneration in bronchioles	0	7.5	10	10	10
Congestion	0	9	9.5	10	10
Edema	0	0	1	4.5	10
Bleeding	0	3	4	6.5	10
Macrophages in alveolus	0	3	4	8	5
Cellular infiltration in respiratory bronchioles	0	7.5	10	10	10

[a]Total of 10 animals per group; the frequency of a less severe but significant lesion was rated at 0.5.
Source: Data taken from Uemura et al. 1995.

thelial space of the bronchioles was observed 14 d postexposure. A summary of microscopic lesions observed in the lungs of exposed mice in presented in Table 4–4. There were no observed exposure-related changes in clinical chemistry indices, in the nasal cavity, or in the major bronchi.

TABLE 4–4 Summary of Lung Weights and Prevalence of Microscopic Lesions in Lungs of Mice Exposed at 15 ppm Diborane for 4 Hours[a]

	Time After Exposure				
End Point	Control	Immediate	1 d	3 d	14 d
Lung/body weight ratio	0.57[b] 0.54[c]	0.74[e] (130)[d]	0.83[e] (146)	0.8[e] (156)	0.63[e] (111)
Microscopic Finding					
PMN neutrophils at bronchiolus	0	10	5	0	0
Macrophages in alveolus	0	2	9	9	0
Inflammatory epithelial degeneration of the bronchioles	0	0	10	10	0
Edema and congestion	0	0	10	10	0
Peribronchiolar thickening	0	0	0	0	10
Subepithelial inflammatory cellular infiltration	0	0	0	0	10

[a]Total of 10 animals per group.
[b]Controls sacrificed immediately after exposure.
[c]Controls sacrificed after 14 days.
[d]Number is parenthesis is the percentage of controls.
[e]Statistically different from controls: $p<0.01$.
Source: Data taken from Uemura 1996.

Nomiyama et al. (1995) investigated the effects of acute and repeated diborane exposures in 5-wk-old male ICR mice. Exposures were conducted in an exposure chamber, and diborane concentrations were measured at 1-min intervals with a toxic gas monitor. For the acute study, groups of 10 mice were exposed at 1 or 5 ppm for 1, 2, 4, or 8 h, and control mice were exposed to filtered room air. Mice were sacrificed 3 d after exposure. There were no mortalities, behavioral or neurological signs, or changes in external appearance observed during the study, and no changes in hematol

ogy or clinical chemistry were noted at termination. Absolute lung weight and lung weight relative to body weight was significantly increased in the group of mice exposed at 5 ppm for 8 h (119% and 117% of controls, respectively). Histopathological evaluation indicated a time-response relationship in the inflammatory epithelial degeneration in the bronchioles observed in the 5-ppm groups exposed for 2, 4, or 8 h (4/10, 9/10, and 10/10 animals affected, respectively, versus 0/10 for control and 1-h exposure group). No significant histopathological changes were noted in the groups of mice exposed at 1 ppm.

In a repeated-concentration inhalation exposure study by Nomiyama et al. (1995), groups of 10 mice were exposed to diborane at 0.2 or 0.7 ppm or to filtered room air for 2 or 4 wk (6 h/d, 5 d/wk) and were sacrificed the day after the last exposure. No differences in behavior or external appearance were observed during the study. There were no concentration-related changes in body weight gain or in clinical chemistry or hematology end points. An apparent concentration-response relationship was observed in the slight infiltration of polymorphous neutrophils noted in the 0.2- and 0.7-ppm exposure groups after a 2-wk and 4-wk exposure period. This infiltration was mainly found in the peribronchiolar region.

Uemura et al. (1995) investigated the effects of repeated inhalation exposures of diborane in 5-wk-old male ICR mice. Ten mice per group were exposed in a dynamic exposure chamber at 5 ppm for 6 h/d, 5 d/wk, for 2 or 4 wk, and control mice were exposed to filtered air for the same time periods. Diborane concentrations were measured at 1-min intervals with a toxic gas monitor. No deaths were observed during the study. The mice in the treatment groups exhibited similar but less frequent signs that were observed in an acute exposure study (face washing movements, restlessness, ruffled fur, and systemic tremor). The 4-wk treatment groups exhibited a slight decrease in body weight 2 and 4 d after exposure (94% and 95% of controls, respectively), and both treatment groups had significant increases in lung weight (164% and 204% of controls, respectively). There were no clinical chemistry changes related to treatment, and hematology changes were minimal. The respiratory histopathological lesions included mucous exudate and inflammatory cells in the nasal cavity, macrophage and plasma cells in the alveolus, lymphoid hyperplasia in the perivascular and peribronchial regions, and pulmonary congestion and edema. The observed hyperplasia and desquamation of Clara cells were more severe in the 4-wk exposure groups than in the 2-wk exposed groups.

3.2.4. Guinea pigs

Comstock et al. (1954) exposed 10 guinea pigs to diborane at 0.8–1.7 mg/m^3 (0.7–1.5 ppm) for 6 h/d, 5 d/wk, for up to 6 mo. No control animals were used. All animals survived 95 exposures, and pathological examinations of the animals did not reveal any abnormalities. The Comstock et al. study has been criticized for its lack of control data, limited pathological data (especially the lack of changes in the rats and guinea pigs), and lack of evidence of the cause of mortality in the rats (EPA 1988).

3.3. Developmental and Reproductive Effects

Groups of 12 14-wk-old male Wistar rats were exposed at 0.11 or 0.96 ppm for 8 wk (6 h/d, 5 d/wk) in exposure chambers (Nomiyama et al. 1996). Diborane concentrations were measured at 1-min intervals using a toxic gas monitor. The animals were killed the day after the last exposure. Both testes from each animal were examined, and sperm from the head plus body and tail of the right epididymis were examined and counted. There were no significant findings in the testes from animals in any of the treatment groups (Nomiyama et al. 1996). A study by Shen et al. (1994, as cited in Uemura [1996] and Nomiyama et al. [1996]) found that mice exposed at 0.7 ppm for 4-wk developed sperm abnormalities and testicular toxicity.

3.4. Genotoxicity

The mutagenic potential of diborane was assessed on *S. typhimurium* TA98, TA100, TA1535, and TA1537 and *E. coli* WP2 *uvrA* following a 2 h exposure at 25 °C using a gas sampling bag. Diborane was diluted with helium in one gas sampling bag, and the appropriate bacterial plate was place in another gas sampling bag. To perform the exposure, the gas sampling bag containing the bacterial plate was filled with the appropriate concentration of the diluted gas. Diborane was mutagenic at concentrations of 0.2–0.5% (v/v; 2,000–5,000 ppm) to TA98 and TA100 with or without metabolic activation and to WP2 *uvrA* at a concentration of 0.5% (5,000 ppm) with metabolic activation (Araki et al. 1994).

3.5. Carcinogenicity

No data were found in the literature concerning the carcinogenic potential of diborane in animals.

3.6. Summary

Inhalation exposure lethality data were available for dogs, rats, mice, rabbits, and hamsters, and nonlethal toxicity data were available for dogs, rats, and mice (see tables 4–5 and 4–6, respectively). The 4-h LC_{50} values ranged from 40 ppm to 80 ppm in rats and from 29 ppm to 31.5 ppm in mice. Younger rats were more sensitive to lethality, having LC_{50} values of approximately 41 ppm compared with older rats' range of 50–80 ppm. The lung was the target organ of diborane toxicity, and death was generally from pulmonary edema. Pathological changes resulting from acute diborane exposures included pulmonary edema, congestion, and hemorrhage. Recent studies in rats and mice have also uncovered the development of multifocal and/or diffuse inflammatory epithelial degeneration in the bronchioles following exposure to diborane. Pulmonary changes produced by exposure to nonlethal concentrations were completely reversible in rats by 2 wk after an acute exposure, and these lesions were being repaired in the mouse by 2 wk postexposure. These signs of toxicity and repair of pulmonary lesions following acute exposure to nonlethal concentrations in animals were similar to the human case reports. In addition to pulmonary changes, some laboratory animals also showed effects in the kidneys and liver.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

Diborane quickly hydrolyzes in water to produce boric acid, hydrogen, and heat and should undergo the same reaction in the lungs. No studies were found in the literature specifically addressing the metabolism and disposition of diborane in humans or animals.

TABLE 4–5 Summary of Acute Lethal Inhalation Data in Laboratory Animals

Species	Concentration (ppm)	Exposure	Time	Effect Reference
Dog	350	15 min	Death—pulmonary edema	Kunkel et al. 1956
Dog	40–125	2–2.5 min	Death—pulmonary edema (2/3)	Kunkel et al. 1956
Rat	159–182	15 min	Calculated LC_{50}	Krackow 1953
Rat	50	4 h	Calculated LC_{50}	Krackow 1953
Rat[a]	40, 42	4 h	Calculated LC_{50}	Jacobson and Lawson 1962
Rat[b]	65	4 h	Calculated LC_{50}	Jacobson and Lawson 1962
Rat[c]	80, 74	4 h	Calculated LC_{50}	Jacobson and Lawson 1962
Rat	158–446	1 h	Death (22/24)	Comstock et al. 1954
Rat	159	2 h	Death (3/6)	Comstock et al. 1954
Rat	228	2 h	Death (6/6)	Comstock et al. 1954
Rat	47	4 h	Death (2/6)	Comstock et al. 1954
Rat	60–140	4 h	Death (46/54)	Comstock et al. 1954
Mouse	29	4 h	Calculated LC_{50}	Jacobson and Lawson 1962
Mouse	31.5	4 h	Calculated LC_{50}	Uemura et al. 1995
Mouse	15	8 h	Death—pulmonary damage (1/10)	Uemura et al. 1995
Rabbit	Unknown	Until death	Death—pulmonary edema	Kunkel et al. 1956
Hamster	50–1,000	Until death	Death—pulmonary edema	Stumpe 1960

[a]Two month EBF rat.
[b]Five month EBF rat.
[c]Five month Wistar (CRDL) rat.

TABLE 4–6 Summary of Acute Nonlethal Inhalation Data in Laboratory Animals

Species	Concentration (ppm)	Duration	Effects[a]	References
Dog[b]	6–14	45 min to 4 h	No blood pressure effects, normal electrocardiograms, slightly increased respiration Postmortem: severe pulmonary hemorrhage and slight edema	Kunkel et al. 1956
Dog[b]	53–63	15 min	Increased rate and depth of respiration, drop in blood pressure, bradycardia Postmortem: Pulmonary congestion, hemorrhage, edema; other organs showed congestion	Kunkel et al. 1956
Dog[b]	125	30 min	Increased small intestine peristalsis and EEG; all activity returned to normal within 1 h.	Kunkel et al. 1956
Rat	0.9 9.2	4 h	3 d postexposure BALF: Concentration-dependent increase in total protein, α_1-antitrypsin activity, and total phospholipids; increases in LDH, neutrophils, and lymphocytes; concentration-dependent decrease in macrophages Serum: α_1-antitrypsin activity increased in the 9.2 ppm group Histopathology: Inflammatory epithelial degeneration in the bronchioles in the 9.2 ppm group	Nomiyama 1995

Species	Concentration (ppm)	Duration	Effects[a]	References
Rat	20	4 h	Immediately after, 1 d, 3 d, or 14 d postexposure: BALF: Neutrophils, total phospholipids, activities of α_1-antitrypsin and superoxide dismutase initially increased; all returned to normal by 14 days postexposure except a significant decrease in α_1-antitrypsin by 14 days Histopathology: Bronchial polymorphonuclear neutrophil infiltration immediately after exposure, becoming milder by 1 and 3 d postexposure; inflammatory epithelial degeneration in the bronchioles 3 d postexposure; returning to normal by 14 d postexposure	Nomiyama 1995
Mouse	1	1, 2, 4, 8 h	3 d postexposure: No observable effects	Nomiyama et al. 1995
Mouse	5	1, 2, 4, 8 h	3 d postexposure: Lung weight: Increased in 8-h group Histopathology: Time-response relationship for inflammatory epithelial degeneration (0/10, 4/10, 9/10, 10/10, respectively)	Nomiyama et al. 1995
Mouse	15	1, 2, 4, 8 h	Observations: all groups—face washing, restlessness; 4-and 8-h groups—ruffled fur, systemic tremors 3 d postexposure: Weights: Liver, kidney, spleen, thymus, body weight	Uemura et al. 1995

			decreased; Lung, trachea weights increased (4- and 8-h groups most affected) Histopathology: Time-response relationship for inflammatory epithelial degeneration (8/10, 10/10, 10/10, 10/10, respectively); longer exposed mice had cellular infiltration in bronchioles, congestion, edema, bleeding	
Mouse	15	4 h	Observations: face washing, restlessness; 3 d and 14 d postexposure groups also had ruffled fur, hypoactivity Immediately, 1 d, 3 d, or 14 d postexposure: Weights: Lung weights increased in all groups; body weight decreased 1 d postexposure Histopathology: Polymorphonuclear neutrophil infiltration of bronchiolar lumens immediately after exposure; macrophages in alveolar and bronchiolar lumen immediately after, becoming more prominent 1 and 3 d postexposure; inflammatory epithelial degeneration, edema, congestion present 1 and 3 d postexposure; reversed by 2 wk postexposure 2 wk postexposure: Histopathology: Inflammatory epithelial degeneration replaced by peribronchiolar thickening; subepithelial inflammatory cellular infiltration	Uemura 1996

[a]Diborane delivered by exposure in a chamber, except for dogs.
[b]Diborane delivered by intratracheal instillation.

4.2. Mechanism of Toxicity

Although originally postulated that the production of boric acid from the hydrolysis of diborane was responsible for toxicity, lethal concentrations of diborane would produce only nonlethal levels of boric acid (Stumpe 1960). The half-life of diborane in a condition of saturated humidity at room temperature was reported to be 0.8–1.5 h (Nippon Sanso Co. 1986, as cited in Nomiyama et al. [1995]). It has been postulated that the heat produced by the highly exothermic reaction of diborane hydrolysis would produce local damage in the lungs (Nomiyama et al. 1995), although this has not yet been proven. It is likely that the mechanism of toxicity is due to direct interaction of diborane with cellular components, especially since diborane is such a potent reducer. There appears to be a similar mechanism of toxicity between species because the respiratory tract has consistently been the target organ, and the cause of death from diborane exposure has always been from pulmonary damage, including edema, hemorrhage, and congestion.

4.3. Structure-Activity Relationships

The use of structure-activity relationships was not necessary for derivation of inhalation exposure guidelines for diborane.

4.4. Other Relevant Information

4.4.1. Species Variability

There did not appear to be much variation between species in sensitivity to lethal concentrations of diborane, and the cause of death from these exposures was a consequence of pulmonary damage including congestion, hemorrhage, and edema. The 4-h LC_{50} values determined by different authors for mice and rats were all within a factor of 2.8 (ranging from 29 ppm to 80 ppm). Mice were more sensitive than rats in lethal and nonlethal studies.

4.4.2. Confounding Factors

Many acute inhalation experiments in animals were conducted in the 1950s and 1960s. A problem that was frequently encountered when using rodents as a test species during this time period was the prevalence of chronic murine pneumonia. This posed a problem when investigating the response following inhalation exposure to toxic compounds, because one could not be certain that the observed response was truly reflective of exposure to the toxicant. Indeed, Jacobson and Lawson (1962) pointed out that lack of subtle pulmonary changes in their study was probably a consequence of the infection. Fortunately, more recent studies have been conducted investigating the toxicity of diborane, thereby increasing the confidence in the data. Despite the presence of pneumonia in mice used in the study reported by Jacobson and Lawson (1962), the 4-h LC_{50} value for mice was the same as that of the study conducted by Uemura et al. (1995). When considering all of the 4-h LC_{50} values derived for mice and rats by the various groups over 42 y, the numbers vary only by a factor of 2.8.

4.4.3. Concentration-Exposure Duration Relationship

The experimentally derived exposure values are scaled to AEGL time frames using the concentration-time relationship given by the equation $C^n \times t = k$, where C=concentration, t=time, and k is a constant. The values of the exponent n generally are in the range of 1–3.5 and "should always be derived empirically from acute inhalation toxicity experiments, in which both the concentration and exposure period are variables" (ten Berge 1986). To calculate n for diborane, a regression plot of the EC_{50} values was derived from the studies by Nomiyama et al. (1995) and Uemura et al. (1995) investigating 1-, 2-, and 4-h exposures at 1, 5, or 15 ppm, with multifocal and/or diffuse inflammatory epithelial degeneration in the bronchioles as the end point of toxicity (see Appendix B). Although n values have generally been derived using lethality data, it was considered appropriate in this case to use the nonlethal pulmonary changes. Toxicity studies demonstrated that the lung remained the target organ at all concentrations of exposure, and the biological response remained the same, becoming more severe with increasing concentration until death occurred from anoxia

as a consequence of severe pulmonary changes. From the regression analysis, the derived value of n=1 was used in the temporal scaling of all the AEGL values ($C^1 \times t = k$; Haber's law).

5. DATA ANALYSIS FOR AEGL-1

5.1. Human Data Relevant to AEGL-1

The only quantifiable human data available was the reported odor threshold range of 2–4 mg/m^3 (1.8–3.6 ppm). Case reports of accidental workplace exposures did not contain information about exposure concentration and duration.

5.2. Animal Data Relevant to AEGL-1

There were no animal data relevant for the derivation of an AEGL-1.

5.3. Derivation of AEGL-1

An AEGL-1 value was not recommended because the AEGL-2 value is below the odor threshold of diborane and no other data pertaining to end points relevant to AEGL-1 definition were available. Absence of an AEGL-1 does not imply that exposure below the AEGL-2 is without adverse effects.

6. DATA ANALYSIS FOR AEGL-2

6.1. Human Data Relevant to AEGL-2

No human data relevant to the derivation of the AEGL-2 were found in the available literature.

6.2. Animal Data Relevant to AEGL-2

Data relevant to the AEGL-2 were available based on several end

points of toxicity following acute exposure to diborane in animals. Nomiyama et al. (1995) and Uemura et al. (1995) found severe inflammatory epithelial degeneration in the bronchioles in mice 3 d following exposures to diborane at 5 ppm for 2, 4, or 8 h (4/10, 9/10, 10/10) and at 15 ppm for 1, 2, 4, or 8 h (8/10, 10/10, 10/10, and 10/10). Many of the mice exposed at 15 ppm also had other respiratory changes, including pulmonary bleeding, congestion, edema, and increased lung and tracheal weights (Uemura et al. 1995). In another study, mice exposed at 15 ppm for 4 h also developed inflammatory epithelial degeneration in the bronchioles by 3 d postexposure. These lesions were replaced by peribronchiolar thickening by 14 d postexposure, and infiltration of lymphocytes into the subepithelial space of the bronchioles was noted (Uemura 1995). Data on rats following acute inhalation exposure to diborane were also available; however, those data indicate that the mouse is a more sensitive model.

6.3. Derivation of AEGL-2

The AEGL-2 values were based on reversible histological changes in the lungs in male ICR mice following a 2-h acute inhalation exposure to diborane at 5 ppm. No effects were observed in mice exposed at 5 ppm for 1 h, while exposure at 5 ppm for 2 h resulted in 4/10 mice developing multifocal and/or diffuse inflammatory epithelial degeneration in the bronchioles (Nomiyama et al. 1995). Studies have demonstrated that these lesions are reversible. There were no other treatment-related changes, such as changes in behavior or appearance, body or organ weight, or in hematological or clinical chemistry indices. A total uncertainty factor (UF) of 10 was applied to the AEGL-2 value. An interspecies UF of 3 was applied because the most sensitive species, the mouse, was used, and the end point of toxicity, reversible histological changes in the lungs, was the most sensitive end point. Further support of a value of 3 is that signs of toxicity and repair of pulmonary lesions following acute exposure to nonlethal concentrations in animals were consistent with the human response reported by case reports. There appears to be a similar mechanism of toxicity between species because the cause of death from diborane exposure has always been from pulmonary damage, including edema, hemorrhage, and congestion. An intraspecies UF of 3 was applied because using the default UF of 10 generates AEGL values that are inconsistent with existing empirical data. For example, the derived 1-h AEGL-2 value is 1.0 ppm with a total UF of 10. Mice exposed at 1 ppm for up to 8 h exhibited no effects of diborane

exposure (Nomiyama et al. 1995). Mice exposed at 0.7 ppm for 6 h/d, 5 d/wk for up to 4 wk developed only slight pulmonary infiltration of polymorphous neutrophils (Nomiyama et al. 1995) and rats exposed at 0.96 ppm for 6 h/d, 5 d/wk for 8 wk developed changes in bronchoalveolar lavage fluid that were not accompanied by histopathological changes (Nomiyama et al. 1996). The use of a higher UF would result in AEGL values that would be below concentrations causing effects in any species for an end point that is supposed to be disabling or cause irreversible effects in a human population.

The experimentally derived exposure value was then scaled to AEGL time frames using the concentration-time relationship given by the equation $C^n \times t = k$, where C=concentration, t=time, and k is a constant (ten Berge 1986). The value of n=1 was used for the temporal scaling based on the derivation presented in Section 4.4.3. and Appendix B.

Although it is considered appropriate to extrapolate from a 2-h exposure to a 10-min exposure duration, the 10-min value of 6.0 ppm approaches that of the 10-min AEGL-3 value of 7.3 ppm. Therefore, the 10-min value was set equal to the 30-min value. AEGL-2 values are presented in Table 4–7.

These values are supported by another study in which the next highest LOAEL for inflammatory epithelial degeneration in the bronchioles in mice was identified as 15 ppm for a 1-h exposure (Uemura et al. 1995).

7. DATA ANALYSIS FOR AEGL-3

7.1. Human Data Relevant to AEGL-3

There were no human data appropriate for derivation of an AEGL-3 found in the available literature. It should be noted that no reports of human mortality following diborane exposure were found in the available literature.

7.2. Animal Data Relevant to AEGL-3

Lethality data were available for several species including dog, rat, mouse, rabbit, and hamster. The 4-h LC_{50} values for the rat ranged from 40

ppm to 80 ppm, and 15-min LC_{50} values for the rat ranged from 159 ppm to 182 ppm. The mouse was slightly more sensitive to lethality than the rat, with 4-h LC_{50} values of 29 and 31.5 ppm.

TABLE 4–7 AEGL-2 Values For Diborane (ppm [mg/m3])

10 min	30 min	1 h	4 h	8 h
2.0 (2.2)	2.0 (2.2)	1.0 (1.1)	0.25 (0.28)	0.13 (0.14)

7.3. Derivation of AEGL-3

A 4-h LC_{50} study in mice was calculated by Uemura et al. (1995). This was a well-defined study in which the mortality ratios at each dose were given. An LC_{01} value could then be estimated by a log-probit analysis of these data. The estimated LC_{01} value of 9.2 ppm was used in the derivation of the AEGL-3. A total uncertainty factor (UF) of 10 was applied to the AEGL-3 value. Because there was little observed variation between species in sensitivity to lethal concentrations of diborane, an interspecies UF of 3 was applied. The 4-h LC_{50} values determined by different authors for mice and rats were within a factor of 2.8 (4-h LC_{50} values ranged from 29 ppm to 31.5 ppm in mice and from 40 ppm to 80 ppm in rats). The lung was the target organ in all species tested, and the biological response remained the same, becoming more severe with increasing concentrations until death occurred from anoxia as a consequence of severe pulmonary changes. An intraspecies UF of 3 was applied because using the default UF of 10 generates AEGL values that are inconsistent with existing empirical data. For example, the derived 1-h AEGL-3 value is 3.7 ppm with a total UF of 10. Mice exposed at 5 ppm for up to 4 h developed only inflammatory epithelial degeneration in the bronchioles, with exposure for 8 h resulting in increased lung weights (Nomiyama et al. 1995). Mice exposed at 15 ppm for 4 h developed pulmonary changes including edema, congestion, and inflammatory epithelial degeneration that were generally resolved or in the process of being resolved within 14 d postexposure (Uemura 1996). The use of a higher UF would result in AEGL values that would be below concentrations causing effects in any species for an end point that is supposed to be life-threatening in a human population.

The experimentally derived exposure value was then scaled to AEGL

time frames using the concentration-time relationship given by the equation $C^n \times t = k$, where C=concentration, t=time, and k is a constant (ten Berge 1986). The value of n=1 was used for the temporal scaling based on the derivation presented in Section 4.4.3. and Appendix B. The 10-min AEGL-3 value was set equal to the 30-min value of 7.3 ppm because the NAC considers it inappropriate to extrapolate from the exposure duration of 4 h to 10 min. AEGL-3 values are presented in Table 4–8.

TABLE 4–8 AEGL-3 Values For Diborane (ppm [mg/m3])

10 min	30 min	1 h	4 h	8 h
7.3 (8.0)	7.3 (8.0)	3.7 (4.1)	0.92 (1.0)	0.46 (0.51)

8. SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity End Points

A summary of the AEGL values for diborane is provided in Table 4–9. Derivation of an AEGL-1 was not recommended. Absence of an AEGL-1 does not imply that exposure below the AEGL-2 is without adverse effects. The AEGL-2 was based on the lowest diborane concentration at which histopathological changes become evident in male ICR mice. These lesions consisted of multifocal and/or diffuse severe inflammatory epithelial degeneration in the respiratory bronchioles. The AEGL-3 value was based on a 4-h LC_{50} study in male ICR mice. Using the data from this study, an LC_{01} value was calculated, and this LC_{01} value was used to derive the AEGL-3.

A useful way to evaluate the AEGL values in context of existing empirical data is presented in Figure 4–1. For this plot, the toxic response was placed into severity categories. The severity categories fit into definitions of the AEGL health effects: no effects, discomfort, disabling, some lethality (an experimental concentration at which some of the animals died), and lethal (100% mortality). The effects that place an experimental result into a particular category vary according to the spectrum of data available on a specific chemical and the effects from exposure to that chemical. The doses often span a number of orders of magnitude, especially when human data exist. Therefore, the concentration is placed on a log scale. The graph

in Figure 4–1 plots the diborane AEGL values along with the existing acute animal toxicity data for diborane in terms of the categories assigned to them. From this plot, one sees that the AEGL values are below any exposure concentration in animals resulting in any effects and should therefore be protective of human health.

TABLE 4–9 Summary of AEGL Values (ppm [mg/m3])

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-1[a] (Nondisabling)	NR	NR	NR	NR	NR
AEGL-2 (Disabling)	2.0 (2.2)	2.0 (2.2)	1.0 (1.1)	0.25 (0.28)	0.13 (0.14)
AEGL-3 (Lethal)	7.3 (8.1)	7.3 (8.1)	3.7 (4.1)	0.92 (1.0)	0.46 (0.51)

[a]Absence of an AEGL-1 does not imply that exposure below the AEGL-2 is without adverse effects.
Abbreviation: NR, not recommended.

8.2. Comparisons with Other Standards and Guidelines

Standards and guidance levels for workplace and community exposures are listed in Table 4–10. The 1-h AEGL-2 value of 1.0 ppm is the same as the ERPG-2 value, and the 1-h AEGL-3 value of 3.7 ppm is similar to the ERPG-3 value of 3 ppm. The 30-min AEGL-3 value of 7.3 ppm is below the IDLH value of 15 ppm. The IDLH was established in 1994 and is based upon the rat 15-min LC_{50} value reported in the Krackow (1953) study. The AEGL-3 is based upon a 4-h rat LC_{01} calculated using the Uemura et al. (1995) study.

8.3. Data Adequacy and Research Needs

Data were not available for derivation of an AEGL-1. Although the odor threshold could be considered relevant to an AEGL-1 because the odor is considered repulsive, the AEGL-2 value is below the odor threshold and this end point is therefore not appropriate. No other human or animal

TABLE 4–10 Extant Standards and Guidelines for Diborane

	Exposure Duration				
Guideline	10 min	30 min	1 h	4 h	8 h
AEGL-1	NR	NR	NR	NR	NR
AEGL-2	2.0 ppm	2.0 ppm	1.0 ppm	0.25 ppm	0.13 ppm
AEGL-3	7.3 ppm	7.3 ppm	3.7 ppm	0.92 ppm	0.46 ppm
ERPG-1 (AIHA)[a]			NA		
ERPG-2 (AIHA)			1 ppm		
ERPG-3 (AIHA)			3 ppm		
PEL-TWA (OSHA)[b]					0.1 ppm
IDLH (NIOSH)[c]		15 ppm			
REL-TWA (NIOSH)[d]					0.1 ppm
TLV-TWA (ACGIH)[e]					0.1 ppm
MAK (Germany)[f]					Not established at the present
MAC (the Netherlands)[g]					0.1 mg/m^3 0.1 ppm

[a]ERPG (emergency response planning guidelines) (AIHA 1996) The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing other than mild, transient adverse health effects or without perceiving a clearly defined objectionable odor. An ERPG-1 for diborane was not appropriate based on the fact that the odor is detectable at a concentration recommended for the ERPG-2. The ERPG-2 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing or developing irreversible or other serious health effects or symptoms that could impair an individual's ability to take protection action. The ERPG-2 for diborane is based on the dog exposure data (Kunkel et al. 1956) that suggest that no effects other than minor irritation should be expected from exposure at 1 ppm for 1 h. Exposure at a nominal concentration

of 6 ppm for 45 min caused effects likely attributable to minor irritation. Detection of the odor by more sensitive members of the population might be expected at this concentration (Krackow 1953). The ERPG-3 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing or developing life-threatening health effects. The ERPG-3 for diborane is half the value causing respiratory toxicity, but not death, in one dog exposed for 45 min, and slightly more than one-third to one-fifth the level causing respiratory toxicity, but not death, in several dogs exposed for 4 h (Kunkel et al. 1956). This concentration is one-tenth to one-twenty-fifth the LC_{50} in mice and rats exposed for 4 h.

[b]OSHA PEL-TWA (permissible exposure limits-time-weighted average) (OSHA 1996) is defined analogous to the ACGIH TLV-TWA, but is for exposures of no more than 10 h/d, 40 h/wk.

[c]IDLH (immediately dangerous to life and health) (NIOSH 1994, 1999) represents the maximum concentration from which one could escape within 30 min without any escape-impairing symptoms or any irreversible health effects. The IDLH for diborane is based on acute inhalation toxicity data in animals (15-min LC_{50} in rats of 159–181 ppm) (Krackow 1953).

[d]NIOSH REL-TWA (recommended exposure limit-time-weighted average) (NIOSH 1999) is defined analogous to the ACGIH TLV-TWA.

[e]ACGIH TLV-TWA (Threshold Limit Value-time-weighted average) (ACGIH 1991, 2000) is the time-weighted average concentration for a normal 8-h workday and a 40-h workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

[f]MAK (Maximale Argeitsplatzkonzentration [Maximum Workplace Concentration]) (Deutsche Forschungsgemeinschaft [German Research Association] 1999) is defined analogous to the ACGIH TLV-TWA.

[g]MAC (Maximaal Aanvaaarde Concentratie [Maximal Accepted Concentration]) (SDU Uitgevers [under the auspices of the Ministry of Social Affairs and Employment], The Hague, The Netherlands 2000) is defined analogous to the ACGIH TLV-TWA.

data pertaining to end points relevant to the AEGL-1 definition were available.

Human case reports of accidental workplace exposure to diborane report reversible signs and symptoms of exposure including chest tightness, shortness of breath and dyspnea, wheezing, nonproductive cough, and precordial pain. However, nothing is known about the actual exposure concentrations. Data in animals have shown concentration-dependent respiratory effects including reversible histological respiratory lesions and pulmonary edema, hemorrhage, and/or congestion leading to death. These

signs of toxicity and repair of pulmonary lesions following acute exposure to nonlethal concentrations in animals were consistent with the human response reported by case reports. Therefore, the animal data are considered appropriate for development of an AEGL-2. Uncertainties remain about interindividual variabilities in the toxic response to diborane, but the category plot (Figure 4–1) demonstrates that the AEGL values should be protective.

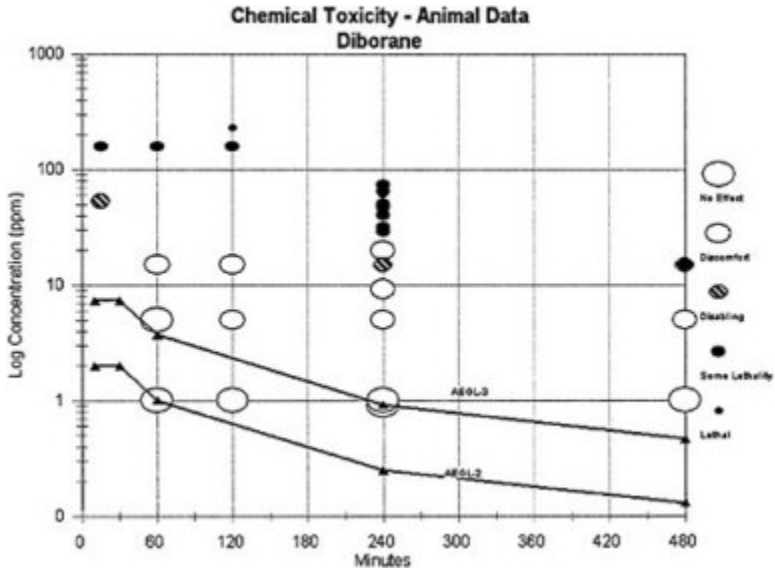


FIGURE 4–1 Category plot of animal toxicity data compared to AEGL values.

Information about the lethality of diborane in humans was not available. Lethality data from animals were considered appropriate for development of an AEGL-3 because the lung was the target organ in all species tested, and the biological response remained the same, becoming more severe with increasing concentrations until death occurred from anoxia as a consequence of severe pulmonary changes. Uncertainties remain about interindividual variabilities in the toxic response to diborane, but the category plot (Figure 4–1) demonstrates that the AEGL values should be protective.

9. REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 1991. Documentation of the Threshold Limit Values and Biological Exposure Indices: (Diborane), 6th Ed. ACGIH, Cincinnati, OH.

ACGIH (American Conference of Governmental Industrial Hygienists). 2000. Threshold Limit Values and Biological Exposure Indices: (Diborane). ACGIH, Cincinnati, OH.

AIHA (American Industrial Hygiene Association). 1996. The AIHA 1996 Emergency Response Planning Guidelines and Workplace Environmental Exposure Level Guidelines Handbook. AIHA, Fairfax VA.

Amoore, J.E., and E.Hautala. 1983. Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. J. Appl. Toxicol. 3(6):272–290.

Araki, A., T.Noguchi, F.Kato, and T.Matsushima. 1994. Improved method for mutagenicity testing of gaseous compounds by using a gas sampling bag. Mutation Res. 307:335–344.

Braker, W., and A.L.Mossman. 1980. Diborane. Pp. 219–222 in: Matheson Gas Data Book, Sixth Edition. Secaucus, NJ: Matheson Gas Products.

Budavari, S., M.J.O'Neil, A.Smith, P.E.Heckelman and J.F.Kinneary, eds. 1996. The Merck Index, 12th Ed. Rahway, NJ: Merck & Co., Inc.

Chemical Economics Handbook. 1996. Boron minerals and chemicals. SRI Interactional.

Comstock, C.C., L.Feinsilver, L.H.Lawson, and F.W.Oberst. 1954. Inhalation toxicity of diborane in dogs, rats, and guinea pigs. (Chem. Corp. Med. Lab. Research Report No. 258.) Army Chemical Center, MD: Chemical Corps Medical Laboratories.

Cordasco, E.M., R.W.Cooper, J.V.Murphy, and C.Anderson. 1962. Pulmonary aspects of some toxic experimental space fuels. Dis. Chest. 41:68–74.

EPA (U.S. Environmental Protection Agency). 1988. Research and Development: Reportable Quantity Document for Diborane.

German Research Association (Deutsche Forschungsgemeinschaft). 1999. List of MAK and BAT Values, 1999. Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Report No. 35. Federal Republic of Germany: Wiley-VCH.

Jacobson, K.H., and L.H.Lawson. 1962. The effect of age or weight on the toxicity of diborane. Toxicol. Appl. Pharmacol. 4:215–219.

Krackow, E.H. 1953. Toxicity and health hazards of boron hydrides. A.M.A. Arch. Induct. Hyg. Occup. Med. 8:335–339.

Kunkel, A.M., E.F.Murtha, A.H.Oikemus, D.E.Stabile, J.P.Saunders, and J.H. Wills. 1956. Some pharmacologic effects of diborane. A.M.A. Arch. Indust. Health. 13:346–351.

Lockheed Martin Energy Systems, Inc. 1988. MSDS for Diborane. Air Liquide America Corporation, Houston, TX.

Lowe, H.J., and G.Freeman. 1957. Boron hydride (borane) intoxication in man. A.M.A. Arch. Indust. Health. 16:5233–533.

Ministry of Social Affairs and Employment (SDU Uitgevers). 2000. Nationale MAC (Maximum Allowable Concentration) List, 2000. The Hague, The Netherlands.

Nippon Sanso Co. 1986. The resolution analysis of diborane. Technical Report, Nippon Sanso Co.

NIOSH (National Institute for Occupational Safety and Health). 1994. Documentation for Immediately Dangerous to Life or Health Concentrations (IDLHS). National Institute for Occupational Safety and Health, Cincinnati, OH. PB94195047, National Technical Information Service, Springfield, VA.

NIOSH (National Institute for Occupational Safety and Health). 1999. NIOSH Pocket Guide to Chemical Hazards. NIOSH Publication 94–116, U.S. Department of Health and Human Services; U.S. Government Printing Office, Washington, DC.

Nomiyama, T. 1995. Inhalation toxicity of diborane in rats assessed by bronchoalveolar lavage examination. Arch. Toxicol. 70:43–50.

Nomiyama, T., K.Omae, C.Ishizuka, K.Hosoda, Y.Yamano, H.Nakashima, T. Uemura, and H.Sakurai. 1996. Evaluation of the subacute pulmonary and testicular inhalation toxicity of diborane in rats. Toxicol. Appl. Pharmacol. 138:77–83.

Nomiyama, T., K.Omae, T.Uemura, H.Nakashima, T.Takebayashi, C.Ishizuka, K.Yamazaki, and H.Sakurai. 1995. No-observed-effect level of diborane on the respiratory organs of male mice in acute and subacute inhalation experiments. J. Occup. Health. 37:157–160.

OSHA. 1996. Limits for Air Contaminants. CFR, Title 29, Part 1910.1000, Table Z-1, p. 16.

Rousch, G. 1959. The toxicology of the boranes. J. Occup. Med. 1:46–52.

Rozendaal, H.M. 1951. Clinical observations on the toxicology of boron hydrides. A.M.A. Arch. Ind. Hyg. Occup. Med. 4:257–260.

Rudolph, R.W. 1978. Boron hydrides, heteroboranes, and their metallo derivatives. In: Kirk-Othmer Encyclopedia of Chemical Technology, 3rd Ed. New York, NY: John Wiley and Sons, Inc.

Shen, M.S., K.Omae, T.Uemura, T.Nomiyama, C.Ishizuka, K.Mori, and H. Sakurai. 1994. Effects of diborane on sperm in mice [in Japanese]. Jpn. J. Ind. Health. 36:28–29.

Stumpe, A.R. 1960. Toxicity of diborane in high concentrations. A.M.A. Arch. Ind. Health. 21:519–524.

ten Berge, W.F. 1986. Concentration-time mortality response relationship of irritant and systemically acting vapours and gases. J. Hazard. Mat. 13:301– 309.

Uemura, T. 1996. Development of pulmonary lesions following acute exposure to diborane in male ICR mice. J. Occup. Health. 38:71–79.

Uemura, T., K.Omae, H.Nakashima, H.Sakurai, K.Yamazaki, T.Shibata, K. Mori, M.Kudo, H.Kanoh, and M.Tati. 1995. Acute and subacute inhalation toxicity of diborane in male ICR mice. Arch. Toxicol. 69:397–404.

Appendixes

APPENDIX A

Derivation of AEGL Values

Derivation of AEGL-1

An AEGL-1 value was not derived because it was not appropriate. Absence of an AEGL-1 does not imply that exposure below the AEGL-2 is without adverse effects. The AEGL-2 value is below the odor threshold of diborane and no other data pertaining to end points relevant to AEGL-1 definition were available.

Derivation of AEGL-2

Key study:	Nomiyama et al. (1995)
Toxicity end point:	LOAEL for inflammatory epithelial degeneration in the bronchioles in male ICR mice (4/10) was 5 ppm for 2 h.
Scaling:	$C^1 \times t = k$ (based on concentration and exposure relationships in Nomiyama et al. [1995] and Uemura et al. [1995]).
Uncertainty factors:	Total uncertainty factor: 10 Interspecies: 3 Intraspecies: 3
Calculations:	$(C/\text{uncertainty factors})^n \times t = k$ $([5\ \text{ppm}]/10)^1 \times 2\ \text{h} = 1\ \text{ppm}\cdot\text{h}$
10-min AEGL-2:	Although it is considered appropriate to extrapolate from a 2-h exposure to a 10-min exposure duration, the 10-min value of 6.0 ppm approaches that of the 10-

	min AEGL-3 value of 7.3 ppm. Therefore, the 10-min value was set equal to the 30-min value.
	10-min AEGL-2=(20 ppm)/10=2 ppm
30-min AEGL-2:	$C^1 \times 0.5$ h=1 ppm·h C^1=2 ppm C=2 ppm
1-h AEGL-2:	$C^1 \times 1$ h=1 ppm·h C^1=1 ppm C=1 ppm
4-h AEGL-2:	$C^1 \times 4$ h=1 ppm·h C^1=0.25 ppm C=0.25 ppm
8-h AEGL-1:	$C^1 \times 8$ h=1 ppm·h C^1=0.125 ppm C=0.13 ppm

DERIVATION OF AEGL-3

Key study:	Uemura et al. (1995)
Toxicity end point:	The data (mortality ratios versus concentration) used to generate a 4-h LC_{01} in male ICR mice were given in the study. Based on those data, an LC_{01} value in mice was calculated to be approximately 9.2 ppm.
Scaling:	$C^1 \times t = k$ (based on concentration and exposure relationships in Nomiyama et al. [1995] and Uemura et al. [1995]).
Uncertainity factors	Total uncertainty factor: 10 Interspecies: 3

	Intraspecies: 3
Calculations:	(C/uncertainty factors)$^n \times t = k$
	([9.17 ppm]/10)$^1 \times 4$ h=3.668 ppm·h
10-min AEGL-3:	Inappropriate to scale from 4 h to 10 min; the 10-min value was set equal to the 30-min value.
	10-min AEGL-3=7.3 ppm
30-min AEGL-3:	$C^1 \times 0.5$ h=3.668 ppm·h
	C^1=7.336 ppm
	C=7.3 ppm
1-h AEGL-3:	$C^1 \times 1$ h=3.668 ppm·h
	C^1=3.668 ppm
	C=3.7 ppm
4-h AEGL-3:	$C^1 \times 4$ h=3.668 ppm·h
	C^1=0.917 ppm
	C=0.92 ppm
8-h AEGL-3:	$C^1 \times 8$ h=3.668 ppm·h
	C^1=0.4585 ppm
	C=0.46 ppm

APPENDIX B

Time Scaling Calculations

The relationship between dose and time for any given chemical is a function of the physical and chemical properties of the substance and the unique toxicological and pharmacological properties of the individual substance. Historically, the relationship according to Haber (1924), commonly called Haber's law (NRC 1993a) or Haber's rule (i.e., $C \times t = k$, where C=exposure concentration, t=exposure duration, and k=a constant) has been used to relate exposure concentration and duration to effect (Rinehart and Hatch 1964). This concept states that exposure concentration and exposure duration may be reciprocally adjusted to maintain a cumulative exposure constant *(k)* and that this cumulative exposure constant will always reflect a specific quantitative and qualitative response. This inverse relationship of concentration and time may be valid when the toxic response to a chemical is equally dependent upon the concentration and the exposure duration. However, an assessment by ten Berge et al. (1986) of LC50 data for certain chemicals revealed chemical-specific relationships between exposure concentration and exposure duration that were often exponential. This relationship can be expressed by the equation $C^n \times t = k$, where n represents a chemical specific, and even a toxic-end-point specific, exponent. The relationship described by this equation is basically the form of a linear regression analysis of the log-log transformation of a plot of C versus t. Ten Berge et al. (1986) examined the airborne concentration *(C)* and short-term exposure duration *(t)* relationship relative to death for approximately 20 chemicals and found that the empirically derived value of n ranged from 0.8 to 3.5 among this group of chemicals. Hence, these workers showed that the value of the exponent n in the equation $C^n \times t = k$ quantitatively defines the relationship between exposure concentration and exposure duration for a given chemical and for a specific health effect end point. Habers rule is the special case where n=1. As the value of n increases, the plot of concentration versus time yields a progressive decrease in the slope of the curve.

To calculate n for diborane, a regression plot of the EC_{50} values was derived from the studies by Nomiyama et al. (1995) and Uemura et al. (1995) investigating 1-, 2-, and 4-h exposures at 1, 5, or 15 ppm, with multifocal and/or diffuse inflammatory epithelial degeneration in the bronchioles as the end point of toxicity. Although n values have generally been

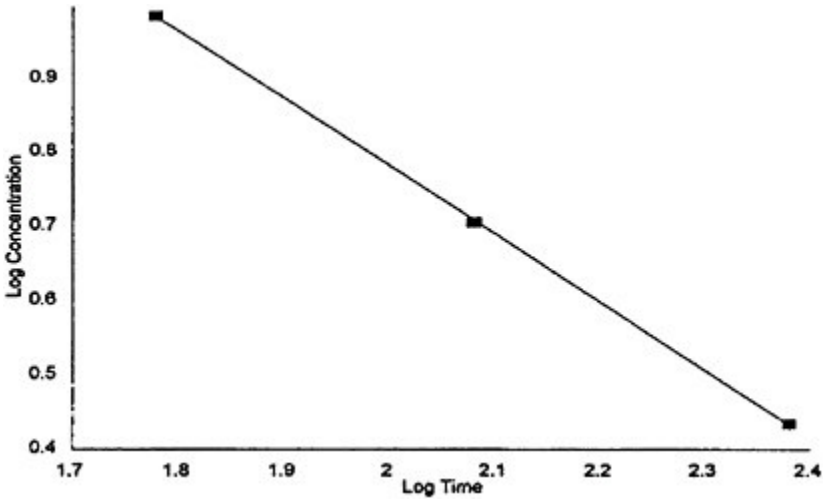
derived using lethality data, it was considered appropriate in this case to use the nonlethal pulmonary changes. Toxicity studies demonstrated that the lung remained the target organ at all concentrations of exposure, and the biological response remained the same, becoming more severe with increasing concentration until death occurred from anoxia as a consequence of severe pulmonary changes. EC_{50} values were derived by probit analysis of the data, and were then analyzed using a linear regression analysis of the log-log transformation of a plot of *C* versus *t* to derived a value of *n* for diborane.

The derived EC_{50} for the 1-, 2-, and 4-h exposures were 9.68, 5.07, and 2.72 ppm, respectively.

Linear regression analysis of plot of log-log transformation of plot of C versus t:

Time (min)	Concentration	Log Time	Log Concentration
240	2.72	2.3802	0.4346
120	5.07	2.0792	0.7050
60	9.68	1.7782	0.9859

n=1.09



Regression plot of EC_{50} values—concentration versus time.

APPENDIX C

AEGL-1

10 min	30 min	1 h	4 h	8 h
NR	NR	NR	NR	NR

Data adequacy: Although the odor threshold could be considered relevant to an AEGL-1 because the odor is considered repulsive, the AEGL-2 value is below the odor threshold and this end point is therefore not appropriate. No other human or animal data pertaining to end points relevant to the AEGL-1 definition were available. Absence of an AEGL-1 does not imply that exposure below the AEGL-2 is without adverse effects.

Abbreviation: NR, not recommended.

AEGL-2

10 min	30 min	1 h	4 h	8 h
2.0 ppm	2.0 ppm	1.0 ppm	0.25 ppm	0.13 ppm

Key reference: Nomiyama, T., Omae, K., Uemura, T., Nakashima, H., Takebayashi, T., Ishizuka, C., Yamazaki, K., and Sakurai, H. 1995. No-observed-effect level of diborane on the respiratory organs of male mice in acute and subacute inhalation experiments. J. Occup. Health. 37:157–160.

Test species/strain/gender/number: Young male ICR mice, 10 per exposure group.
Exposure route/concentrations/durations: Inhalation; 5 ppm for 1, 2, 4, or 8 h.
Effects: 5 ppm: 1 h, no effects; 2 h, inflammatory epithelial degeneration in the bronchioles (4/10); 4 h, inflammatory epithelial degeneration in the bronchioles (9/10); 8 h, inflammatory epithelial degeneration in the bronchioles (10/10).
End point/concentration/rationale: 5 ppm for 2 h resulted in reversible inflammatory epithelial degeneration in the bronchioles.
Uncertainty factors/rationale:
Total uncertainty factor: 10
Interspecies: 3—An interspecies UF of 3 was applied because the most sensitive species, the mouse, was used, and the end point of

toxicity, reversible histological changes in the lungs, was the most sensitive end point. Further support of a value of 3 is that signs of toxicity and repair of pulmonary lesions following acute exposure to nonlethal concentrations in animals were consistent with the human response reported by case reports. There appears to be a similar mechanism of toxicity between species because the cause of death from diborane exposure has always been from pulmonary damage, including edema, hemorrhage, and congestion.

Intraspecies: 3—An intraspecies uncertainty factor of 3 was applied because using the default uncertainty factor of 10 generates AEGL values that are inconsistent with existing empirical data. For example, the derived 1-h AEGL-2 value is 1.0 ppm with a total uncertainty factor of 10. Mice exposed at 1 ppm for up to 8 h exhibited no effects of diborane exposure (Nomiyama et al 1995). Mice exposed at 0.7 ppm for 6 h/d, 5 d/wk for up to 4 wk developed only slight pulmonary infiltration of polymorphous neutrophils (Nomiyama et al. 1995) and rats exposed at 0.96 ppm for 6 h/d, 5 d/wk for 8 wk developed changes in bronchoalveolar lavage fluid that were not accompanied by histopathological changes (Nomiyama et al. 1996). The use of a higher uncertainty factor would result in AEGL values that would be below concentrations causing effects in any species for an end point that is supposed to be disabling or cause irreversible effects in a human population.

Modifying factor: Not applicable

Animal to human dosimetric adjustment: Not applicable

Time acaling: $C^n \times t = k$ where n=1; based on a regression plot of the EC_{50} values derived from the studies by Nomiyama et al. (1995) and Uemura et al. (1995) investigating 1-, 2-, and 4-h exposures at 1, 5, or 15 ppm, with multifocal and/or diffuse inflammatory epithelial degeneration in the bronchioles as the end point of toxicity. Although it is considered appropriate to extrapolate from a 2-h exposure to a 10-min exposure duration, the 10-min value of 6.0 ppm approaches that of the 10-min AEGL-3 value of 7.3 ppm. Therefore, the 10-min value was set equal to the 30-min value.

Data adequacy: Human case reports of accidental workplace exposure to diborane report reversible signs and symptoms of exposure including chest tightness, shortness of breath and dyspnea, wheezing, nonproductive cough, and precordial pain. However, nothing is known about the actual exposure concentrations. Data in animals have shown concentration and time dependent respiratory effects including reversible histological respiratory lesions and pulmonary edema, hemorrhage, and/or congestion leading to death. These signs of toxicity and repair of pulmonary lesions following acute

exposure to nonlethal concentrations in animals were consistent with the human response reported by case reports. Therefore, the animal data are considered appropriate for development of an AEGL-2. Uncertainties remain about interindividual variabilities in the toxic response to diborane, but the category plot (Figure 4–1) demonstrates that the AEGL values should be protective.

AEGL-3

10 min	30 min	1 h	4 h	8 h
7.3 ppm	7.3 ppm	3.7 ppm	0.92 ppm	0.46 ppm

Key reference: Uemura, T., Omae, K., Nakashima, H., Sakurai, H., Yamazaki, K., Shibata, T., Mori, K., Kudo, M., Kanoh, H., and Tati, M. 1995. Acute and subacute inhalation toxicity of diborane in male ICR mice. Arch. Toxicol. 69:397–404.

Test species/strain/gender/number: Young male ICR mice, 10 per exposure group.

Exposure route/concentrations/durations: Inhalation: 0, 11.3, 22.1, 35.1, 37.7, 44.8 ppm for 4 h.

Effects:

Concentration	*Mortality*
11.3 ppm	0/10
22.1 ppm	3/10
35.1 ppm	3/10
37.7 ppm	9/10
44.8 ppm	9/10

LC_{50}: 31.5 ppm (provided in reference)

LC_{01}: 9.17 ppm (calculated by log-probit analysis)

End point/concentration/rationale: 9.17 ppm for 4 h was the calculated LC_{01}, which is the threshold for lethality, a defined end point for the AEGL-3.

Uncertainty Factors/Rationale:

Total uncertainty factor: 10

Interspecies: 3—An interspecies uncertainty factor of 3 was applied because there did not appear to be much variation between species in sensitivity to lethal concentrations of diborane. The 4-h LC_{50} values determined by different authors for mice and rats were within a factor of 2.8 (4-h LC_{50} values ranged from 29 ppm to 31.5 ppm in mice and from 40 ppm to 80 ppm in rats). The lung was the target organ in all species tested, and the biological response remained the same, becoming more severe with increasing concentrations until death occurred from anoxia as a consequence of severe pulmonary changes.

Intraspecies: 3—An intraspecies uncertainty factor of 3 was applied because using the default uncertainty factor of 10 generates AEGL values that are inconsistent with existing empirical data. For example, the derived 1-h AEGL-3 value is 3.7 ppm with a total uncertainty factor of 10. Mice exposed at 5 ppm for up to 4 h developed only inflammatory epithelial degeneration in the bronchioles, with exposure for 8 h additionally resulting in increased lung weights (Nomiyama et al. 1995). Mice exposed at 15 ppm for 4 h developed pulmonary changes including

edema, congestion, and inflammatory epithelial degeneration that were generally resolved or in the process of being resolved within 14 d postexposure (Uemura 1996). The use of a higher uncertainty factor would result in AEGL values that would be below concentrations causing effects in any species for an end point which is supposed to be life-threatening in a human population.
Modifying factor: Not applicable
Animal to human dosimetric adjustment: Not applicable
Time Scaling: $C^n \times t = k$ where n=1; based on a regression plot of the EC_{50} values derived from the studies by Nomiyama et al. (1995) and Uemura et al. (1995) investigating 1-, 2-, and 4-h exposures at 1, 5, or 15 ppm, with multifocal and/or diffuse inflammatory epithelial degeneration in the bronchioles as the end point of toxicity. The 10-min AEGL-3 value was set equal to the 30-min value of 7.3 ppm because the NAC considers it inappropriate to extrapolate from the exposure duration of 4 h to 10 min.
Data adequacy: Information about the lethality of diborane in humans was not available. Lethality data from animals were considered appropriate for development of an AEGL-3 because the lung was the target organ in all species tested, and the biological response remained the same, becoming more severe with increasing concentrations until death occurred from anoxia as a consequence of severe pulmonary changes. Uncertainties remain about interindividual variabilities in the toxic response to diborane, but the category plot (Figure 4–1) demonstrates that the AEGL values should be protective.